

A novel chromosomal anomaly involving rearrangement of the *MLL* gene in a case of acute myelogenous leukemia. *K. Chun*¹, *D. Bouman*¹, *P. Chiang*¹, *M. Minden*², *M. Shago*³. 1) Dept. of Pathology, University Health Network, Toronto, Canada; 2) Dept. of Medical Oncology, University Health Network, Toronto, Canada; 3) Dept. of Pathology, Mount Sinai Hospital, Toronto, Canada.

Objectives: Initial G banding analysis of the peripheral blood from a 50-year old AML patient with monocytic features yielded a karyotype of 47,XY,+8,del(11)(q21q23). Since the presence of an 11q23 breakpoint involving the *MLL* gene can have prognostic implications, molecular cytogenetic studies were undertaken in order to characterize the abnormality in detail.

Methods: FISH analysis was performed on previously G banded metaphases using *MLL*, WCPs 9 and 11 and M-FISH probes. RT-PCR analysis of mRNA was undertaken to look for the presence of the *MLL-AF9* fusion transcript.

Results: Metaphase FISH analysis using a dual colour break apart *MLL* probe revealed that the 5' portion of the *MLL* gene had been inserted into 9p22, while the 3' region remained on chromosome 11. WCP and M-FISH experiments confirmed that part of chromosome 11 had been inserted into 9p and demonstrated that the rest of the deleted segment of 11q had not been transferred elsewhere in the genome. With this information, the karyotype was assigned as 47,XY,+8,der(9)ins(9;11)(p22;q23q23),del(11)(q21q23). RT-PCR on mRNA was positive for the *MLL-AF9* fusion transcript.

Conclusions: FISH analysis revealed a cryptic rearrangement involving the *MLL* gene at 11q23. RT-PCR confirmed that the cryptic rearrangement in this patient leads to the fusion of *MLL* and *AF9* on the der(9) chromosome. The classic t(9;11)(p22;q23) is a recurrent aberration associated with AML M5, which results in the fusion of the *MLL* gene and the *AF9* gene on the der(11) chromosome. *MLL* rearrangements are generally predictive of poor clinical outcome. However, the prognosis for children and adults with AML and the t(9;11), especially with the presence of trisomy 8, is associated with a more favorable outcome. The presence of the *MLL-AF9* transcript is consistent with the clinical phenotype of this patient.

Complete Characterization of Medulloblastoma Cell Lines: New Chromosomal Regions Potentially Involved in the Pathogenesis of This Tumor. *S. Dube*^{1, 2}, *M. Muleris*³, *F. Fortin*¹, *Y. Théorêt*², *N. Lemieux*^{1, 2}. 1) Dept. of Pathology and Cell Biology, University of Montreal, Montreal, Canada; 2) Research Center, Ste-Justine Hospital, Montreal, Canada; 3) Institut Curie, Paris.

Medulloblastoma (MB), a primitive neuroectodermal tumor of the cerebellum, represents approximately 25% of all intracranial tumors in children. Several studies showed numerous non-random chromosomal abnormalities that may play a role in the occurrence, the development and the spreading of this cancer. However, the specific oncogenic events involved in the development and progression of this tumor remain unknown. In this study, conventional karyotyping (CT), fluorescence in situ hybridization (FISH) and comparative genomic hybridization (CGH) were performed to allow a more comprehensive cytogenetic evaluation of three new MB cell lines: HSJ-MB1, HSJ-MB2 and UM-MB1. CT and FISH analyses of the three cell lines revealed DNA polyploidy and multiple complex chromosomal rearrangements resulting in gains of 7q, 8q and 11q and losses of 3p, 4q, 9p, 10q, 11p and 14. Using CGH, we validated CT and FISH results and detected 3q, 7q, 8q, 11q, 20p and 20q as over-represented regions and 4, 10 and 11p as under-represented regions. The combined analysis of all results shows many clonal imbalances which have previously been reported such as gains of 7q and 8q and losses of 3p, 9p, 10q and 11p. Those changes are described in the majority of PNET/MB tumors. Furthermore, some novel changes involving chromosomal segments 3q, 4p, 4q, 14q, 20p and 20q were revealed by our analyses. These recurrent chromosomal imbalances found in our cell lines might involve genes implicated in the tumorigenic process of MBs. Thus, some other candidate genes could be involved in addition to those already described. The results presented here demonstrate that the three new MB cell lines analyzed are cytogenetically representative of the PNET/MB tumors and should be helpful to further our understanding of the biology of these tumors. However, additional studies are needed to furnish more insight on the roles played by the chromosomal changes observed. This shows how essential it is to characterize completely medulloblastoma cell lines.

Unusual variants of the t(15;17) rearrangement and their implications in the diagnosis/prognosis of acute promyelocytic leukemia. C. Astbury, L. Christ, M. Lemos, M. Kochera, S. Schwartz. Department of Genetics and Center for Human Genetics, Case Western Reserve University and University Hospitals of Cleveland, Cleveland, OH.

Acute promyelocytic leukemia (APL), a cancer with potentially lethal coagulopathy necessitating accurate and rapid diagnosis, is characterized by the t(15;17)(q22;q21), and generally responds well to retinoid treatment. The common translocation leads to the production of a PML-RARa fusion protein, which prevents the normal function of the RARa receptor, leading to leukemogenesis. While the t(15;17) can be seen cytogenetically in the majority of patients, it is estimated that approximately 2% of APL is due to cryptic rearrangements between the PML and RARa genes. We report two unusual variants of the translocation that have important implications in the diagnosis of APL and demonstrate the impact of FISH on diagnoses and treatment. In the first patient, who presented with AML M3, the t(15;17) was not seen cytogenetically. However, FISH analysis with the Vysis dual color probe revealed an atypical fusion pattern. This rearrangement was characterized as ins(17;15)(q21.1;q22q22), with the typical yellow fusion signal on one chromosome 17 (instead of on chromosome 15). Reports cite a potential resistance to all-*trans* retinoic acid (ATRA) treatment with this cryptic rearrangement. In the second patient, who presented with AML, the common t(15;17) was seen cytogenetically. However, confirmation studies using FISH revealed an extra red signal (from the PML gene) on the der(17), as well as the yellow fusion signal on the der(15). In this patient, the PML gene had been broken in either the bcr 2 or 3, instead of the more common bcr 1. Patients with breakpoints in either of bcr 2 or 3 also may have reduced sensitivity to ATRA treatment. The studies of these two patients have important implications in the diagnosis and evaluation of APL. (1) It emphasizes the importance of FISH analysis in the diagnosis of APL, with potential implications for treatment and prognosis; and (2) it demonstrates that standard cytogenetics may not always be sufficient, leading to an underdiagnosis of cryptic rearrangements.

Chromosome 11 rearrangements and specific MLL amplification disclosed by spectral karyotyping in a RAEB patient. *G. Calabrese*^{1,2}, *D. Fantasia*^{1,2}, *E. Morizio*^{1,2}, *P.M. Toro*^{1,2}, *V. Gatta*², *P. Guanciali Franchi*^{1,2}, *M. De Cinque*^{1,2}, *A. Spadano*³, *L. Stuppia*^{1,4}, *G. Palka*^{1,2}. 1) Dip. S. Biomed/Genetica Medica, Univ G D'Annunzio, Chieti, Italy; 2) S. Genetica U., Pescara Hospital, Pescara, Italy; 3) Dip Ematologia, Pescara Hospital, Pescara, Italy; 4) Ist. Citomorfologia N&P, CNR Chieti, Italy.

A patient with refractory anaemia with excess of blasts (RAEB) had a complex karyotype 48,XY,del(3)(q13),t(5;13)(q13;q12?),-11,+3mar as defined by GTG banding technique. The karyotype was refined by spectral karyotype (SKY) analysis which classified the three markers as der(11). FISH analysis using specific probes for the MLL gene showed a complete decoration of the three der(11) while no YAC clone flanking MLL region was found on the markers. Fibre-FISH analysis using single-colour cosmid MLL probe (Appligene-Resnova, Italy) and green-red dual colour large MLL probes (Vysis-Olympus, Italy) disclosed the presence of repeated MLL-specific signals with a direct duplication pattern and confirmed isolated MLL-specific amplification. RT-PCR on RNA from bone marrow cells was also performed using specific primers scattered along MLL transcript. This analysis revealed normal size MLL products which appeared very abundant when compared to those from unaffected individuals, showing that amplification involved the whole MLL gene. To our knowledge this is the first example of isolated MLL gene amplification in a RAEB patient. He survived six months after diagnosis supporting clinical adverse effect of MLL amplification. Moreover present results further demonstrate the usefulness of SKY analysis in detecting cryptic chromosome rearrangements and disclosing the mechanism of disease progression.

HER-2/neu FISH in breast cancer: Is it the gold standard? *S. Gokhale, Z. Gatalica, A. Mohammad, A. Rampy, G.R.V.N. Velagaleti.* Department of Pathology, University of Texas Medical Br, Galveston, TX.

Overexpression of HER-2/neu oncogene in breast cancer patients is correlated with disease free survival (DFS) and overall survival (OS). The most commonly used methods for the detection of HER-2/neu status are immunohistochemistry (IHC) and FISH. However, there is a lot of controversy with regard to the best method of detecting the status of HER-2/neu. Several studies have been published with mostly contradicting results with some advocating FISH as gold standard while others IHC. Also, most of the studies chose arbitrary cut-off levels for positive results (10%) and have no validation. In order to address all these issues, we designed a retrospective pilot study of 40 samples with known IHC status. The samples were selected in such a way as to represent all 4 categories of IHC scores equally (10 samples each in 0, 1+, 2+ and 3+). FISH was performed using Vysis Pathvysion™ probe. For validation, 5 cases of reduction mammoplasty were analyzed using same protocols. Two independent scorers in a blinded fashion did scoring. Our results showed significant discordance between FISH and IHC in 3 of the 4 categories. FISH showed HER-2/neu amplification in 2/8 cases with IHC score of 0, 7/10 cases with IHC score of 1+, 3/10 cases with IHC score of 2+ and one false positive case with IHC score of 3+. The rate of discordance was much higher in the 0 and 1+ categories compared to published literature. This could be due to the lower cut-off rates for positive amplification established by validation in our study (6.5% vs 10%). Another reason could be the amplification of HER-2/neu in DCIS, which was not taken into consideration by IHC. DCIS with amplification of HER-2/neu has a higher probability of becoming invasive and hence could be a prognostic marker. Our analysis showed that FISH positive and IHC negative patients have a poor prognosis in terms of DFS and OS compared to FISH negative and IHC negative patients. Further, our results also showed that IHC in comparison to FISH has a comparable specificity (98%), but has a very low sensitivity (46%). Based on these results, we consider FISH to be the gold standard for detecting HER-2/neu status in breast cancer.

CGH analysis of 34 cases of colorectal carcinoma. *S. Cheng*¹, *R. Tang*². 1) Department of Anatomy, College of Medicine, Chang Gung University, Kweishan, Taoyuan, Taiwan; 2) Colorectal Section, Chang Gung Memorial Hospital, Linkou, Taiwan.

The carcinogenesis procedure of colorectal carcinoma has been well studied. We are particularly interested in the chromosomal changes on the genomic level in the various Dukes' stages. To broaden the spectrum of the examined region to the whole genome we used the method of comparative genomic hybridization (CGH). Of 34 collected cases of colorectal adenocarcinoma, there are a case of stage A, 15 cases of stage B, 15 cases of stage C, and 3 case of stage D, with 33 cases demonstrating copy number alternation. The DNA are extracted from both normal and tumor tissues from patients, and labeled by nick translation with digoxigenin-11-dUTP and biotin-16-dUTP, respectively. The equivalent amount of both DNA hybridized on the chromosomes of a normal male. The karyotypes and copy number were analyzed with Quips software (Applied Imaging). Among them 1q (32%), 6q (29%), 7p (41%), 7q (41%), 8q (41%), 10p (29%), 13p (29%), 13q (59%), 16q (29%), 20p (50%), 20q (56%) have higher incidence. The chromosomes with higher incidence of the copy number loses are 17p (32%), 18p (29%), and 18q (50%). Comparing the non-metastasis (A and B) with metastasis stages (C and D) the former has more incidence (average 6.6 per chromosome) of losses than the later (average 4.6); however, the later has more incidence of gain (11.9) than the former (8.4). The results may imply that during the tumorigenesis the deletion does occur in some chromosomal region. Nonetheless, the nondisjunction occurs in some chromosomes, and the copy number increases. The 20q is a frequent gained chromosomal region with 19 among 34 cases (56%) reveal a gain. Among them 10 are from 16 non-metastatic stages (63%), 9 are from 18 metastatic stages (50%). The other frequent gained chromosome is 13q (59%), with 8 of 16 (50%) non-metastatic cases, and 12 of 18 (67%) metastatic cases. Each of chromosomes 7p, 7q, and 8q has a gain in 41% (14 in 34) of cases. Most often lost chromosomes are 18q (50%), with 10 in 16 non-metastatic cases, and 7 in 18 metastatic cases. Chromosomes 17p and 18p are deleted in ~30% of cases. (This study is supported by grants NSC89-2320-B-182-092 and CMRP1236 to SDC.).

Detection of Chromosome Aberrations in CLL and Multiple Myeloma Using FISH Probe Panels. *S. Ahsan, A. Wiktor, D. Van Dyke.* Medical Genetics, Henry Ford Hospital, Detroit, MI.

Recent studies have suggested that specific chromosome aberrations have prognostic significance in Chronic Lymphocytic Leukemia (CLL) and Multiple Myeloma (MM). Cytogenetic analysis of metaphase cells is routinely used to identify many of these abnormalities but fluorescent in situ hybridization (FISH) provides a technique to detect lower level mosaicism and small deletions in both metaphase and interphase cells. We investigated the utility of FISH (Vysis, Inc.) panels in 24 CLL and 40 MM patients. Karyotype and FISH were performed on samples from 24 patients with known or suspected CLL. The CLL panel includes probes for D13S319(13q14.3), MLL1(11q23), D12Z3(12 centromere), p53(17p13.1), IGH(14q32). Karyotype and FISH were abnormal in 5/24 (21%) cases, 3(13%) had abnormalities identified by karyotype alone, and 7 (29%) by FISH alone. Karyotype and FISH were normal in 9 patients. Two patients had karyotype abnormalities not included in the panel (one each 20q deletion and loss of Y). One low-level 11q deletion karyotype was not identified by FISH. Seven cases (29%) had a normal karyotype and abnormal FISH. Five cases with abnormal karyotypes were either confirmed or clarified by the FISH studies. For known or suspected MM, 40 cases were analyzed by karyotype and FISH panel. The probes included in the MM panel are Rb1(13q14), p53(17p13.1), MLL1(11q23), IGH/CCND1(t(11;14)). Both karyotype and FISH abnormalities were identified in 4/40 (10%) MM cases, 6 (15%) had abnormalities identified by karyotype alone, and 4 (10%) by FISH alone. Karyotype and FISH were normal in 26 patients. Three patients had 45,X,-Y/46XY karyotype and normal FISH. Two patients had complex abnormalities that were not included in the panel. In one mosaic complex karyotype including 13q deletion, FISH did not identify Rb1 loss. Four patients (10%) had a normal karyotype with abnormal FISH results. Three patients had complex karyotypes and abnormal FISH results confirming the cytogenetic abnormalities. These results indicate that FISH panels improve the detection rate of common chromosomal aberrations in CLL and MM, and are therefore a useful adjunct to routine cytogenetic analysis.

Familial renal carcinoma with novel 3/8 translocation. *H.J. Decker¹, D. Reutzel^{1,2}, E. Lausch², H. Brauch³, M. Holl², B. Zabel².* 1) Medical Genetics, Bioscientia Inst Ltd, Ingelheim, Germany; 2) Moleculargenet. Laboratory Children's Hospital, Johannes-Gutenberg-University of Mainz, Germany; 3) Dr. Margarete Fischer-Bosch-Institute for Clinical Pharmacology, Stuttgart, Germany.

Up to 3% of all renal cell carcinoma (RCC) are inherited. Association of familial renal carcinoma cases with mutations in the VHL (von Hippel-Lindau) tumorsuppressor gene in clear cell RCC and the MET oncogene in papillary RCC have been demonstrated. We analysed a family with 4 members suffering from clear cell renal carcinoma (ccRCC). Molecular studies were done in two patients. Both were found negative for alterations in the VHL gene by DNA sequencing. Cytogenetic evaluation using conventional GTG-banding techniques revealed a reciprocal translocation of chromosome 3q and chromosome 8p whereas the unaffected brother showed a normal 46,XY karyotype. To refine our findings we applied FISH with combined whole chromosome painting (WCP) probes for chromosome 3 and 8. WCP-FISH revealed an additional pericentric inversion of der(3) not seen in conventional cytogenetic studies. A two step event, a reciprocal translocation (3q;8p) and a pericentric inversion of der(3) results in a specific fluorescence pattern seen in both affected patients. The breakpoint described on chromosome 3 is different from the well characterized t(3;8) translocation [t(3;8)(p14.2;q24.1)] segregating with familial clear cell renal carcinoma. Our results suggest that, apart from the known genes on chromosome 3p predisposing for renal carcinoma, there might be additional genes involved in renal tumorigenesis on chromosome 3q.

Estimates of Consensus in Cell Selection and Scoring of FISH Data. *P.E. Barker¹, P. Pinsky², W. Wang², S. Srivastava², D. Hocker¹, X. Wu³, M.R. Spitz³.* 1) DNA Technology Group/Biotechnology Division, NIST, Gaithersburg, MD; 2) Cancer Biomarkers Group, Division of Cancer Prevention, NCI/NIH; 3) Department of Epidemiology, M. D. Anderson Cancer Center, Houston, Texas.

As a collaborating laboratory of the NCI Early Detection Research Network (EDRN), the National Institute of Standards and Technology (NIST) supports validation of assays developed by discovery laboratories of the EDRN. One such assay examines cancer risk by quantifying chromosome breaks in cultured human lymphocytes. In order to standardize criteria for cell selection and classification of chromosome aberrations, a web-based validation study was conducted. Two hundred fluorescence in situ hybridization (FISH) images were collected in CD-R format and assigned random identification numbers. Detailed criteria were established for this NIST FISH Image Set 02-01 based on jointly developed NIST- M. D. Anderson Cancer Center criteria for cell selection, and for classification of metaphases as excluded, normal or abnormal. Eight observers were selected from a randomized pool (N=54) of experienced (> 5 years) volunteers who responded to an email announcement distributed in the cytogenetics community through the AMP and ACF listserves. All eight observers were certified, experienced cytogeneticists. Half were doctoral level and half were BS/MS level laboratory staff. The questions addressed were: 1) Given standard criteria, how similarly did cytogeneticists choose cells and score chromosome damage? and 2) Did concordance vary with educational level, years experience or professional certification? This is the first step toward developing a uniform, fully-validated test system for induced chromosomal damage. Results suggest greater consensus on population chromosome breakage rates than which individual cells of the population were judged adequate for analysis. These results will be used to estimate the components of assay variance that can be ascribed to definitions, technical variables or biological variables. (Supported by Interagency Agreement #Y1CN-0103-01 and CA86390 to MRS).

Unusual chromosomal abnormalities in two cases of malignant lymphoma. *M. Bertoni*¹, *A. Babu*², *V. Mizhiritskaya*², *M.J. Macera*². 1) Department of Pathology; 2) Division of Molecular Medicine and Genetics, Department of Medicine, Wyckoff Heights Medical Center.

An 80-year-old male presented with a right submandibular lymphadenopathy. Physical examination was unremarkable except for slight normochromic normocytic anemia. The tumor had existed for some time and was thought to be inflammatory. A diagnosis of low-grade small cleaved cell malignant lymphoma was established. Cytogenetic analysis of the tumor revealed both numerical and structural rearrangements. All of the abnormalities were non-clonal except for a 45,X,-Y clone. All other complements were seen as single cell abnormalities and include: 47,XY,+Y/ 47,XY,+5/ 47,XY,-1,+2mar/ 47,XY,+X,add(12)(q22),t(14;18)(q32;q21). The t(14;18) translocation appears to be the characteristic rearrangement that places the IGH transcriptional enhancer next to the BCL2 gene. This is believed to be responsible for the over expression of the anti apoptotic BCL2 protein. The remaining cells were normal. The second case, a 49 year old male who presented with fever, night sweats and weight loss, was found to have generalized lymphadenopathy. Biopsy of the lymph node revealed diffuse large B-cell malignant lymphoma. Cytogenetic evaluation revealed an abnormal clone with a near tetraploid complement. The chromosome count is in the range of 81 to 85 and includes structural as well as numerical abnormalities. Missing chromosomes in relation to tetraploidy, are the Y, 1, 6, 9(2), 10, 12, 13, 14, 15, 16 and 17(2). Both X chromosomes are structurally abnormal. The complement also includes 3 to 5 additional unidentified marker chromosomes resulting from structural rearrangements. In these two cases, the complexity and number of chromosome abnormalities is reflective of the severity of the lymphoma. It is recommended that all lymphomas be cytogenetically analyzed for clarification that can provide both an indication of disease progression and a better understanding of these malignant tumors.

Deletions of 11q22-23 and 13q14.3 detected by fluorescence in situ hybridization (FISH) are associated with aggressive clinical course in chronic lymphocytic leukemia (CLL). A.W. Block¹, J.M. Panasiewicz¹, M.L. Hackbarth², J. Ramanarayanan³, A. Chanan-Khan³. 1) Clinical Cytogenetics Lab, Roswell Park Cancer Inst, Buffalo, NY; 2) Vysis, Inc, Downer's Grove, IL; 3) Department of Medicine, Roswell Park Cancer Inst, Buffalo, NY.

Chronic lymphocytic leukemia (CLL) is a malignant disorder characterized by progressive accumulation of functionally incompetent B-lymphocytes. The outcome of the disease is variable and depends upon the clinical stage with median survival ranging from 7-10 years in limited and 2-3 years in advanced stage disease. Genomic aberrations are not yet considered part of the staging system, though various abnormalities have been noted to have clinical significance. Conventional cytogenetic analysis is often unsuccessful due to the low mitotic index of these malignant cells thus yielding limited information. We established a fluorescence in situ hybridization (FISH) - based panel to detect numerical and submicroscopic chromosome abnormalities in non-dividing interphase cells. Mononuclear cells from peripheral blood and bone marrow of 23 patients (14 males, 9 females; median age 60, range 38 - 87 years) were analyzed by FISH for chromosome 12 trisomy, deletions of chromosome bands 11q22-23 (ATM), 13q14-14.3 (Rb1/D13S25/LAMP1), and 17p13 (p53); and translocations involving 14q32 (IgH). Chromosome abnormalities were identified in 14/23 patients (pts). The most frequent changes were mono- and bi-allelic deletions of 13q (11/14 pts) and deletions or rearrangements of 11q (5/14 pts). 4/14 pts had 2 aberrations with 3/4 pts with deletions of both 11q and 13q and 1/4 pts with rearrangement of 11q and 17p deletion. One patient had a 14q32 rearrangement with an unidentified translocation partner. No trisomy 12 was observed. While 13q abnormalities have been associated with long treatment-free intervals, patients with concurrent deletion of 11q and 13q in this study population had chemotherapy-refractory advanced stage disease and poor clinical outcome.

Monoplastic Synovial Sarcoma with Clonal Complex Translocations involving Chromosome number X,2,3,8,15 and 18. *J.H. Lin, I. Akhtar, E. Seguerra, N. Motiwala, L. Freedman, H.O. Shah.* Pathology & Labs, Nassau University Medical Ctr, East Meadow, NY. / SUNY at Stony Brook, Stony Brook, NY.

This 21-year-old woman presented with two rather well circumscribed firm nodules on the postero-lateral aspect of the right elbow measuring up to 5.0 x 4.0 x 4.0 cm. She developed similar nodules at different times since she was 16 years of age for which she underwent surgery four times in Turkey and the lesions were diagnosed as inflammatory fibromatosis. Fine needle aspirate biopsy and immunohistochemistry studies showed small blue cell tumor of low-grade sarcoma, likely of synovial sarcoma. The differential diagnosis included primitive neuroectodermal tumor, Ewings sarcoma, alveolar rhabdomyosarcoma, lymphoma, and malignant melanoma. The follow-up surgical specimen revealed monophasic synovial sarcoma.

Cytogenetic study of the tumor revealed a wide array of clonal complex translocation 46,X,t(2;18;X;8)(p16;q11.2;p11.2;q24.1),t(2;3)(p13;p21)add(15)(p10). Translocation, t(X;18)(p11;q11) involving a transcriptional activation domain on the SYT gene on chromosome 18q11., to a certain degree, helps elucidating proper diagnosis in difficult cases. Abnormalities such as found in this case define, in part, the critical events in soft tumor oncogenesis and may contribute to more accurate diagnosis, prognosis and treatment strategies. To date, this kind of translocation has been seen in each case of fibrosarcoma and malignant fibrous histiocytoma.

Cryptic and complex chromosomal rearrangements and deletion of P53 gene identified by fluorescence in situ hybridization in a patient with Mantle Cell Lymphoma. *S. Li¹, L. Zhang¹, W.F. Kern², M. Gupta³, R.E. Eichner³, F.R. Bates¹, J.J. Mulvihill¹.* 1) Dept Pediatrics, BSEB224, Univ Oklahoma Hlth Sci Ctr, Oklahoma City, OK; 2) Dept Pathology, Univ Oklahoma Hlth Sci Ctr, Oklahoma; 3) Dept Medicine, Univ Oklahoma Hlth Sci Ctr, Oklahoma.

A 50-year old man was referred for an evaluation of chromosomal anomalies associated with a mantle cell lymphoma (MCL). In addition to a translocation between chromosomes 11 and 14 [t(11;14)(q13;q32)] detected by G-banded chromosome analysis, the patient also had a deletion of chromosome 10 [del(10)(q24)], and other unidentifiable, but consistent marker chromosomes in some of his peripheral blood cells. FISH analysis utilizing multiple DNA probes was performed. The patient had complex translocations among five chromosomes; 8, 11, 14, 17, and 21 and a deletion of p53 gene other than t(11;14) and the deletion of chromosome 10. Interestingly both homologues of 14, 17 and 21 were involved, and three chromosomal breaks were occurred on homologues of chromosome 14. Those translocations are cryptic and impossible to detect by conventional cytogenetic studies. Those subtle changes could be unrecognized before but commonly present in the majority of patients with mantle cell lymphoma.

Genetic status of chronic myeloid leukemia patients and the clinical course of disease. *S. Moore, J. Beerworth, A. Hillier, T. Kuczek, M. Nicola, J. Suttle, T. Hughes.* Cancer Cytogenetics Unit, Division of Hematology, Institute of Medical & Veterinary Science, Adelaide, Australia, 5000.

The effect of karyotype, BCR-ABL transcript, ABL-BCR transcript and BCR-ABL D-FISH deletion on presentation characteristics, duration of chronic phase and overall survival of CML patients was examined. The median follow up was 115 months (range 86 - 228 months). Conventional cytogenetic analysis, RT-PCR and D-FISH were performed on 55 presentation CML patient BM samples. Case notes were reviewed for the patients clinical data at presentation, response to therapy, duration of chronic phase and overall survival. An additional 17 cases were analysed by D-FISH because they had either a complex Philadelphia (Ph) rearrangement (bringing the total number in this group to 14) or additional abnormalities at presentation (bringing the total number in this group to 12).

The 55 patients in the original study group all expressed BCR-ABL; 22 had b2a2, 29 had b3a2 and 4 had both transcripts. 30 of the 55 also expressed ABL-BCR. With regard to the parameters studied there was no significant difference between these groups. All patients with ABL-BCR expression lacked D-FISH deletions. Of the 25 ABL-BCR negative patients, 7 had deletions detected by D-FISH. 6/14 patients with a complex Ph contained D-FISH deletions, although none of the 12 patients with additional cytogenetic abnormalities were seen to have a deletion of the der(9). 13 of the patients tested by D-FISH were treated with Imatinib, and of these, 4 developed resistance because of a mutation in the ABL Imatinib binding site. One of the patients with ABL mutation showed a D-FISH deletion.

Neither the type of BCR-ABL fusion transcript nor the presence of ABL-BCR fusion transcript affects clinical parameters at presentation of CML, nor do they mark patients with altered response to therapy or survival times. D-FISH deletions are not seen with greater frequency in patients who show additional abnormalities at presentation, and are more common in patients who have a complex Philadelphia rearrangement.

Complex chromosomal abnormalities in myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) studied by M-FISH. *P.W. Hsu¹, J. Sarkey¹, N. Moy¹, K. Szego¹, H.D. Preisler², A. Raza².* 1) Pediatrics, Rush Medical College, Chicago, IL; 2) Rush Cancer Institute, Rush Medical College, Chicago, IL.

Accurate identification of complex chromosomal abnormalities (CCA) in MDS and AML patients has been hampered by limitations of G-banding analysis (GA). Newly developed M-FISH and SKY technologies have improved the characterization of these complex aberrations. We performed M-FISH analysis on 16 MDS and 6 AML patients who presented with 3 or more chromosomal abnormalities. M-FISH analysis confirmed 71 (40%) and reclassified 94 (53%) abnormalities detected by GA. The reclassified abnormalities included mainly 41 (44 %) add and 36 (38%) mar. The origin of hsr and dmin was identified. M-FISH also revealed 9 new abnormalities. However, 3 abnormalities detected by GA were missed. In characterizing aberrations of chromosomes 5, 7 and 20, 21 of 22 cases (95%) had chromosome 5 rearrangements. Four cases (18%) had del(5q). The remaining 17 cases (81%) had chromosome 5 unbalanced translocations. Six of 17 (35%) cases had 5;17 unbalanced translocations. The other unbalanced chromosome 5 translocations involved chromosomes 13 and 15 in 3 (18%) and 2 (12%) cases, respectively. Moreover, 10 of 17 cases (59%) had different sizes of chromosome 5 segments involved in at least 2 independent unbalanced rearrangements. None of these 21 cases, however, had -5 as the sole chromosomal abnormality. Chromosome 7 abnormalities were detected in 12 of 22 cases (55%). -7 and der(7) were seen in 5 (42%) and 6 cases (50%), respectively. 7q- was only detected in one case (8%). Chromosome 20 abnormalities were observed in 11 of 22 cases (50%). All but one case (91%) had unbalanced translocations, frequently presenting as marker chromosomes. Only one case (9%) had del 20q. Our studies demonstrate that M-FISH significantly enhances the accuracy of the identification and interpretation of CCA. Clearly, complex unbalanced chromosome translocations, particularly involving chromosomes 5, 7 and 20, are the most frequent abnormalities in MDS and AML patients with CCA. The complexity of these changes suggests the presence of chromosomal instability in these patients.

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Relationship between clinical diagnosis and cytogenetic findings in malignant hematological disorders. *M.H. Karimi-Nejad, M. Zanganeh, R. Karimi-Nejad.* PO Box 14665/154, Pathology & Genetics Ctr, Tehran, Iran.

More than 1000 bone marrow samples have been analyzed in our center. 583 cases were referred for malignant disorders, 159 for non malignant, and 258 without diagnosis at the time of sampling. Of the 159 non malignant cases, 50 and 53 for pre and post transplantation of anemia respectively, the 56 remainder for post transplantation of a malignant disorder. The probable clinical diagnosis of malignant cases were: 181 for ALL, 174 AML, 143 CML, 34 MDS, 28 for lymphomas and 26 for other malignancies such as MM, MPD, and CLL. 145/181 of ALL, 163/174 of AML, 130/143 of CML, 33/34 of MDS, 26/28 of lymphoma, 47/50 of anemia and all of pre- or post- bone marrow transplantation cases were conclusive. When we divided these figures into two categories of first 500 and final 500 cases, inconclusive cases have decreased significantly. The decrease in failure rates are most significant in ALL cases, from 28% to 5% and in CML, from 11% to zero. The main reason is better clinical information at time of sample reception, consequent to closer communication with clinicians enabling better culture and handling techniques. Our data indicate that close communication and necessary clinical information is a prerequisite to appropriate culture set up and handling, influencing greatly the rate of culture failure; and also essential for effective cytogenetic analysis of bone marrow spreads.

Cytogenetic study of malignant triton tumor: a case report. *A.L. Hawkins, M.H. Haddadin, D. Depew, P.P. Long, C.A. Griffin.* Departments of Pathology and Oncology, The Johns Hopkins University School of Medicine, Baltimore, Maryland.

Malignant triton tumor (MTT) is a highly malignant neoplasm, classified as a variant of malignant peripheral nerve sheath tumor (MPNST) with rhabdomyoblastic differentiation. Few cytogenetic studies of MTT have been reported using conventional G-band analysis. Here we report a comprehensive cytogenetic study of a case of sporadic MTT using G-banding, Spectral Karyotyping (SKY), and fluorescent in situ hybridization (FISH) for specific regions. A complex hyperdiploid karyotype with multiple unbalanced translocations was observed: 48~55, XY, der(7)add(7)(p?)dup(7)[2], der(7)t(7;20)(p22;?)ins(20;19)[5], der(7)ins(8;7)(?;p22q36)t(3;8)t(8;20)[15], -8[19], r(8)dup(8), +der(8)r(8;22)[4], -9[9], der(11)t(3;11)(?;p15)t(3;19)t(19;20), der(12)t(8;12)(q21;p11), der(13)t(3;13)(p11;q24), -17, -19, der(19)t(17;19)(q11.2;q13.1), -20, -22, +4~7r [cp25] / 46,XY [11] following ISCN 1995 nomenclature where possible. However, note that breakpoints were frequently omitted where only SKY information was known for a small part of an involved chromosome. Most cells also contained multiple acentric fragments and at least one large ring chromosome, which by SKY was shown to be derived from several apparently monosomic chromosomes, including multiple inclusions of chromosome 8. Our analysis revealed some breakpoints in common with previously reported MTT, MPNST and rhabdomyosarcomas (RMS), namely 7p22 and 11p15. FISH study showed high increase of copy number for CMYC and loss of a single copy of TP53, in contrast to the normal copy numbers of both genes most commonly reported in MPNSTs.

Analysis of Chromosomal Imbalances in Korean Ovarian Cancer using Degenerate Oligonucleotide Primed PCR-Comparative Genomic Hybridization. *G.J. Kim¹, J.O. Kim², J.Y. Lee¹, E.K. Hong³, Y.H. Chun¹, S.H. Park¹.* 1) Dept. of Anatomy, Korea University College of Medicine, Seoul, Korea; 2) Dept. of Obstetrics & Gynecology, Eulji University School of Medicine, Seoul, Korea; 3) Dept. of Pathology, Korean National Cancer Center.

Ovarian cancer is the fourth leading cause of cancer death among women in the world and yet its pathogenesis is poorly understood. Little is known about genetic abnormalities in ovarian cancers because of the difficulty to growing them in culture for chromosome studies. Recently, studies using technique of degenerate oligonucleotide primed PCR (DOP-PCR) - comparative genomic hybridization (CGH) permits genetic imbalances screening of the entire genome using only small amounts of tumor DNA. Twenty-three of ovarian cancers were investigated for chromosomal aberrations by DOP-PCR-CGH on microdissected tissues. Twenty-two of ovarian cancers were investigated for chromosomal aberrations by DOP-PCR-CGH on microdissected tissues. The most frequently detected chromosomal gains involved 1q32-qter (32%), 2q31-qter (32%), 3q25-qter (63%), 6p21 (63%), 7q (45%), 8q22-qter (73%), 14q31 (45%), 15q24-qte (45%), 16p (45%), 17q23-qter (90%), 20q (73%), and Xp (54%). The most frequently detected loss of chromosomal materials involved 4q21-q31 (50%), 5q13-q21 (63%), and 13q21 (50%). 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 ovarian cancer.

Identification of chromosomal imbalances in Korea metastatic gastric cancer by degenerate oligonucleotide primed-PCR-comparative genomic hybridization. *J.Y. Lee¹, H.Y. Hwang¹, S.J. Cho², N.H. Won², Y.H. Chon¹, S.H. Park¹.* 1) Dept.of Anatomy, Korea Univ College Med, Seoul, Korea; 2) Dept.of Pathology, Korea Univ College Med, Seoul, Korea.

Gastric cancer is the major cause of cancer deaths in Asian countries, including Korea. Genetic changes during the progression and metastasis of gastric cancer remain unclear. The aim of this study was to analyze chromosomal imbalances in early and advanced metastatic gastric cancers. Comparative Genomic Hybridization (CGH) analysis was used to identify chromosomal imbalances in 12 early and 23 advanced metastatic gastric cancers. In early metastatic gastric cancer, common gains were detected at 6p22-q24 (58%), 4 (58%), 5p15.2-q33 (50%), and 8p21-qter (50%), and common losses were detected at X (42%) and 1p33-pter (25%). In advanced metastatic gastric cancer, common gains were detected at 20q (65%), 8p21-qter (65%), 5 (57%), and 7pter-q31 (52%), and common losses were detected at X (35%), 1p33-pter (30%), 16p (26%), and 17p12-p13 (22%). Chromosomal imbalances identified in this study provide candidate regions that may play some important role in the progression and metastasis of gastric cancers.

Multicolor deconvolution microscopy of thick biological specimens. *C. Maierhofer*¹, *M. Neumann*², *M. Speicher*¹. 1) TU Munich, Institute of Human Genetics, Munich, Germany; 2) Carl Zeiss Vision GmbH, Hallbergmoos, Germany.

One limitation to understanding disease at the cellular level has been the inability to analyze DNA efficiently on a cell by cell basis within the natural tissue context. However, DNA analyses at single cell resolution should be instrumental for the understanding of cancer cell biology, cancer evolution, for genetic mosaic analysis and rare cell events. Here we present a multicolor deconvolution technique for three-dimensional (3D) microscopy, which generates 3D data by optically sectioning the specimen. Images are captured using an epifluorescence microscope equipped with a motorized table to collect a stack of images at defined levels in z-direction. Deconvolution refers to a computational method used to reduce out-of-focus fluorescence in 3D microscopy images. After deconvolution, 3D-reconstruction algorithms are applied. In contrast to other, previous studies we employed up to seven different color channels for probe detection, which allowed the simultaneous analysis of multiple probes within biological specimen with a thickness of up to 30 μ m. In addition, a DNA counterstain is used for volume labeling of the nuclei which offers the opportunity for a simultaneous segmentation of nuclei. Different probe sets, consisting of various chromosome-centromere specific probes or of YAC/BAC-probes consisting of probes that are according to CGH-data of diagnostic or prognostic value for patients with breast or ovarian cancer were applied to normal and ovarian tumor specimen. The technology allows the analysis of tissues at a single-cell resolution and yields new insights into how cells in a tissue become increasingly heterogeneous and disorganized in their structural properties during carcinogenesis. Efforts are under way to use the interphase-FISH data to identify areas which may represent aggressive portions within a tumor. This information may then be used for a guided dissection of specific tissue regions for a "navigated" RNA-sampling are needed in order to improve the predictive power of expression analysis.

An Unusual translocation t(7;21) in a case of thrombocytopenia. *M.J. Macera¹, M. Mistry², P. Chandra¹, A. Babu¹.*
1) Div Mol Med & Genetics, Dept of Med, Wyckoff Heights Med Ctr, Brooklyn, NY; 2) Brookdale Hospital Med Ctr, Brooklyn, NY.

A 64 year old patient presented initially with a hypercellular marrow with granulocytic and megakaryocytic hyperplasia, and fibrosis consistent with myeloproliferative disorder. The platelets were reduced in number with some larger forms. There were nucleated red blood cells with an increase in neutrophils and a shift to the left (occasional metamyelocytes and myelocytes). The final diagnosis was leukocytosis with anemia and thrombocytopenia with leukoerythroblastic changes. Initial cytogenetic analysis of unstimulated peripheral blood, suggested a 46,XX,t(7;21)(p22;q22.1) translocation. A single normal cell was also detected. Due to the subtle nature of the translocation, dual color fluorescence in situ hybridization (FISH) with whole chromosome paints (wcp) for 7 and 21 was done for conformation. Signal for wcp 21 was seen over the entire normal 21 homologue and just a small proximal band on the derivative 21. Signal was also seen at the distal short (p) arms of both chromosome 7s. Signal for wcp 7 was seen over both of the chromosome 7s from qter up to p22, and a small proximal band on the derivative 21. A three probe combination (D21S259:D21S341:D21S342) with overlapping sequences, specific for the region 21q22.13-21q22.2 of chromosome 21 was applied to confirm the breakpoint. Signals were seen on the normal 21, and on both der(7) chromosomes. No signal was detected on the der(21). The karyotype was revised to 46,XX,t(7;21)(p22;q22.1).ish t(7;21)(p22;q22.1)(wcp7+,wcp21+, D21S259:D21S341:D21S342+;wcp7+,wcp21+,D21S259:D21S341:D21S342-),-7,+der(7)t(7;21)(p22;q22.1)(wcp7+,wcp21+,D21S259:D21S341:D21S342+). The most likely origin for this unusual aberration is an initial reciprocal translocation between chromosomes 7 and 21, followed by loss of the normal chromosome 7 and a duplication of the derivative 7, during the progression of the leukemic clone. Although this specific t(7;21)(p22;q22.1) translocation is not common, the acute myeloid leukemia 1 (AML1) gene located at chromosome 21q22, has been implicated in a number of hematopoietic disorders including familial thrombocytopenia.

Frequencies and characterization of cytogenetically unrelated clones in various hematologic malignancies: A seven year experience in a single institution. *J. Han*^{1, 2}, *K.S. Theil*², *G.A. Hoeltge*². 1) Clinical Pathology, Col Med, Dong-A-Univ, Pusan, Korea; 2) Clinical Pathology, The Cleveland Clinic Foundation, Cleveland, OH.

Cytogenetically unrelated clones are uncommon findings in hematologic disorders. In a retrospective review of 1,110 hematologic malignancies with clonal chromosome abnormalities analyzed during the last seven years, we found that 27 (2.4%) patients had karyotypically unrelated clones occurring in the same sample. Unrelated clones were found in 3.5% (7/202) of acute myeloid leukemias (AML), 5.3% (11/206) of myelodysplastic syndromes (MDS), none of 40 acute lymphoblastic leukemias (ALL), 0.4% (1/233) of myeloproliferative disorders (MPD), and 2.6% (8/306) of lymphoproliferative disorders (LPD). Twenty-five patients showed two unrelated clones and two had three unrelated clones. The most consistent clonal chromosome abnormalities were del(5q) (7 cases), +8 (6 cases), del(20q) (5 cases), and del(7q), +12, +21, and 22 (three cases each). Of interest, in nine patients the unrelated clones differed by having numerical or structural aberrations that involved the same chromosomes: three patients presented structural abnormalities of one chromosome in one clone and numerical abnormalities of the same chromosome in the unrelated clone; three showed two different structural aberrations with different breakpoints involving the same chromosomes in two unrelated clones; and the remaining three patients had two different structural chromosome abnormalities involving the same chromosomes and same breakpoints. These findings, together with the high frequency of intermediate clones and unbalanced, secondary-type cytogenetic aberrations, are evidence of karyotype instability. Unrelated clones may originate from a common precursor not recognizable as abnormal at the cytogenetic level through submicroscopic molecular genetic changes and evolutionary processes. Further study of gene expression patterns in cases with unrelated clones may help identify molecular similarities in the clones that appear to be seemingly unrelated at the karyotype level.

Mechanisms of tumorigenesis in mammary tumors from BALB/c-Trp53-heterozygous mice: A model for Li-Fraumeni syndrome. *R. Naeem*¹, *A.C Blackburn*², *M.S Mohammed*⁴, *S.P Naber*³, *D.J Jerry*². 1) Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Department of Veterinary & Animal Sciences, University of Mass. Amherst, MA; 3) Spectral Genomics, Houston, TX; 4) Department of Pathology Baystate Medical Center, Springfield, MA.

Breast cancer is the most common tumor type observed among women with Li-Fraumeni syndrome (LFS). Mammary tumors (MT) are also the most prevalent tumor type in BALB/c-Trp53-heterozygous female mice suggesting a genetic predisposition towards mammary tumorigenesis. The spectrum and histopathology of MT in this background is similar to the LFS patients, and therefore, presents a unique model for the study of breast cancer in LFS. The mechanisms of mammary tumorigenesis were examined in these p53-deficient mice. Loss of the wild type allele of Trp53 (LOH) was detected in the majority of mammary tumors from BALB/c-Trp53-heterozygous females (96%). LOH was also observed in 85% of the lymphomas and sarcomas arising in these mice which was similar to the rate of LOH for mammary tumors ($P=0.35$). To study the mechanism of LOH, normal tissues and tumors from 8 mice were karyotyped using short-term culture methods. FISH was used to further characterize the cytogenetic results. BAC CGH microarrays were applied to corroborate the cytogenetic results and to identify genomic regions undergoing frequent loss and gain. The mammary tumors contained a hypodiploid population of cells lacking one copy of chromosome 11 as well as a near-tetraploid population of cells. The BAC CGH data also indicated loss throughout chromosome 11. These results demonstrate that loss of the wild type allele of Trp53 in these mammary tumors results from missegregation of chromosomes rather than deletions. Preliminary results suggest that recombination within chromosome 11 occurs frequently and precedes loss of heterozygosity.

Acute Promyelocytic Leukemia (APL) with cryptic t(15;17), and atypical morphology demonstrating PML/RAR Alpha-gene transcript by FISH Method. *H.O. Shah¹, L.I. Sheng¹, E. Seguerra¹, M. Macera², H. Shih¹, J.H. Lin¹.* 1) Pathology & Lab Cytogenetics, Nassau Univ Medical Ctr, East Meadow, NY. / SUNY at Stony Brook, Stony Brook, NY; 2) Wyckoff Heights Medical Center, Brooklyn, NY.

This 28-year-old black woman presented to our emergency room for rectal bleeding and heavy menses for past two weeks. The CBC showed WBC 12K/mm³, RBC 3.1 mil./mm³ Hb 9.3g/dl, Hct 27.6%, MCV 88.7 fl and platelet 16K/mm³. There were 7% myeloblasts and 34% of promyelocytes with reniform nuclei in the peripheral smear which showed positive myeloperoxidase stain. No Auer rods were identified. The peripheral blood chromosomal studies were carried out under impression of acute promyelocytic leukemia (FAB, AML-M3) on admission. Shortly after admission, she suddenly developed disseminated intravascular coagulopathy and went into coma with severe upper gastrointestinal bleeding and intracranial hemorrhage. The coagulation tests revealed fibrinogen, 99 mg/dl and D-Dimer, >8.0 microgram/ml < 16.0 micrograms/ml. The patient was then treated with Heparin infusion (300 u/hr) for two days. However, the condition deteriorated and she succumbed on the third hospital day.

Most classic APL shows PML-RAR alpha gene fusion with exceptional cases lacking this change. The resultant chimeric protein is very much responsible for a variety of clinical manifestations and inferences of specific therapeutic strategy for patients with APL. In rare cases, no apparent chromosomal abnormality is seen in chromosomes Nos. 15 or 17. In such cases, FISH study with t(15;17) probe (Vysis) can clinch the accurate diagnosis. In the present case with the apparently normal cytogenetic and atypical morphology on peripheral blood, the diagnosis of APL was confirmed by the FISH technique.

PA-1, a human ovarian teratocarcinoma cell line, demonstrates a single chromosome abnormality, t(15;20)(p11.2;q11.2): analysis with FISH, SKY, and BAC microarray analysis. S.A. Sarraf¹, R. Tejada¹, T. Dennis², M. Oberst¹, K.C. Simon¹, J.K. Blancato¹. 1) Oncology, Georgetown University, Washington, DC; 2) National Human Genome Research Institute, NIH, Bethesda, MD.

Chromosomal changes are associated with altered regulation of oncogenes and tumor suppressors. Cell lines derived from primary tumors contain numerous chromosomal aberrations, which are studied to delineate progressive steps in tumor evolution. The ovarian teratocarcinoma cell line, PA-1, demonstrates a single chromosomal aberration: a reciprocal translocation, t(15;20)(p11.2;q11.2) (Zeuthen et al. 1980). Chromosome 20q amplification is implicated in both breast and ovarian cancer as a poor prognostic marker; this region contains a number of oncogenes including AIB1, STK15, MDM2, ZNF217, and the ovarian tumor marker HE4 (Courjal et al. 1996; Tanner et al. 2000; Bingle et al. 2002). The PA-1 cell line was analyzed in our lab using fluorescence in situ hybridization (FISH), spectral karyotyping (SKY), and BAC microarray analysis. FISH revealed gene amplification of AIB1(20q12) and that STK-15 (20q13) was present in normal copy number. SKY analysis and rev ish after microdissection confirmed the presence of the consistent single change, t(15;20)(p11.2;q11.2). Immunoblot demonstrated 3.6-fold over-expression of the AIB1 protein product, but no elevation of the STK-15. BAC oncogene microarray analysis showed minimal gene amplification of TERC (3q26.3), PAK1 (11q13.5-q14), KRAS2 (12p12.1), ERBB2 (17q21.2), and JUNB (19p13.2). The regulation of AIB1 and/or other oncogenes in the 20q12-13 region, have been affected due to the translocation. This abnormality appears to cause overexpression of the AIB1 or other protein products and may provide a stabilizing or immortalizing effect on the cell line. Further studies are aimed at directed analysis of the translocation breakpoints in the PA-1 cell line. Bingle, L. et al. (2002). *Oncogene* 17(21): 2768-73. Courjal, F. et al. (1996). *British Journal of Cancer* 74: 1984-1989. Tanner, M. M. et al. (2000). *Clinical Cancer Research* 6(5): 1833-9. Zeuthen, J. et al. (1980). *International Journal of Cancer* 25(1): 19-32.

Characterization of Myoepithelial tumors using combined cytogenetic, microarray and immunohistochemical approach. *G.R.V.N. Velagaleti*^{1,2}, *H. Kuivaniemi*^{3,4}, *G. Tromp*⁴, *J. Palazzo*⁵, *K.M. Graves*², *M. Guigneaux*⁶, *T. Wood*⁶, *M. Sinha*⁶, *B. Luxon*⁶, *Z. Gatalica*². 1) Department of Pediatrics, University of Texas Medical Branch, Galveston, TX; 2) Department of Pathology, University of Texas Medical Branch, Galveston, TX; 3) Center for Molecular Medicine & Genetics, Wayne State University, Detroit, MI; 4) Department of Surgery, Wayne State University, Detroit, MI; 5) Department of Pathology, Thomas Jefferson University, Philadelphia, PA; 6) Department of Human Biological Chemistry & Genetics, University of Texas Medical Branch, Galveston, TX.

Myoepithelium is an integral part of the mammary ductal and lobular architecture, positioned between luminal cells and the basement membrane. Myoepithelial hyperplasia frequently accompanies luminal epithelial hyperplasia, but true myoepithelial neoplasms are distinctly rare. The gene expression profile of a benign, pure myoepithelial tumor carrying a reciprocal translocation involving chromosomes 8 and 16 [46,XX,t(8;16)(p23;q21)] was analyzed using Affymetrix® Gene chip®U95AV2. Differential analysis identified 857 genes with 2 or more fold mRNA change in comparison to pooled normal breast control. Expression results were grouped based on the chromosomal location of the genes and protein function. Immunohistochemical analysis (IHC) of 15 such protein products confirmed these results, both in normal and tumor tissues. The predictive value of 3 antigens (GH, ninjurin 1, and CK19) was tested in additional cases of benign and malignant myoepithelial mammary and salivary gland tumors and an identical pattern of protein expression was observed. Additionally, a 23-fold increase in growth hormone mRNA, and 32-fold increase in ionotropic glutamine receptor GluR2 in the tumor indicated the existence of two autocrine growth loops, which were confirmed by IHC. Our results reaffirmed the value of microarray analysis in rapid and comprehensive identification of diagnostically useful tumor markers and identified several potential pathogenetic mechanisms (autocrine and paracrine growth stimuli) in the development of myoepithelial tumors.

MICROMETASTASIS DETECTION IN PERIPHERAL BLOOD FROM BREAST CANCER PATIENTS BY CYTOGENETIC TECHNIQUES. *A.E. Rojas-Atencio¹, M.L Soto-Alvarez¹, K.M. Urdaneta¹, L. Gonzalez², G.R. Garcia¹, F. Alvarez - Nava¹, H. Acevedo².* 1) Unidad de Genetica, Universidad del Zulia, Maracaibo - Zulia, Venezuela; 2) Hospital Clinico, Maracaibo - Zulia, Venezuela.

The identification of micrometastasis in peripheral blood (PB) from cancer patient become an important tool in the management of neoplasia due to is a marker metastasis prognostic. The objective of this study was to investigate the presence of tumoral cells on PB from patient with breast cancer (BC), by standard cytogenetic techniques. Twenty-four and Twelve samples from tumoral tissue and PB from patient with invasive and in situ BC were analyzed, respectively. At least 20 and 50 100 metaphase in tumoral tissue and PB were screened, respectively. Chromosomal abnormalities in 61,11%; patients with invasive BC versus 25%; patients with in situ BC were found. By comparing the chromosomal abnormalities seen in tumoral tissue and PB, 63%; were same in both analyzed samples, this findings was significant statically. In conclusion the identification of chromosomal abnormalities in PB from breast cancer patients, could be considered as micrometastasis, since the presence of early metastasis on subject with this findings and in situ BC it has been reported of this manner, PB cytogenetic analysis in patients with BC could became in other tool to define recurrence risk and death in these patients.

Telomeric Fusion as a Mechanism for the Loss of Chromosome 1p in Meningioma. *J.R. Sawyer^{1,2}, M. Husain², J. Lukacs¹, R. Lichti-Binz¹, O. Al-Mefty³.* 1) Cytogenetics Laboratory, Arkansas Children's Hosp, Little Rock, AR; 2) Department of Pathology, University of Arkansas for Medical Sciences, Little Rock, AR; 3) Department of Neurosurgery, University of Arkansas for Medical Sciences, Little Rock, AR.

Chromosome aberrations in meningiomas are characterized by the primary aberration of monosomy 22 in benign tumors. The most common secondary aberration is the deletion of 1p, which is associated with the progression to atypical and malignant tumors. We report a detailed cytogenetic investigation of chromosome aberrations in a series of 88 meningiomas using Giemsa banding, and multicolor spectral karyotyping (SKY). Clonal chromosome aberrations were identified in 46 (52%) tumors by G-banding. Thirty-five tumors showing complex chromosome aberrations not fully characterized by G-banding were subsequently reanalyzed by SKY. The SKY technique refined the G-band findings in 18 (51%) of the tumors on which it was applied. The most common features of cytogenetic progression in the complex karyotypes were chromosome arm specific losses relating to the formation of deletions and dicentric chromosomes involving chromosome 1p. Part or all of chromosome 1p was lost in 19 tumors. Five tumors showed evidence for the loss of 1p in a progressive step-wise series of telomeric fusions involving the formation of unstable intermediates resulting from breakage-fusion-bridge (BFB) cycles. Five recurring dicentric chromosomes were identified, including dic(1;11)(p11;p11), dic(1;12)(p12~13;p11), dic(1;22)(p11;q12~13), dic(7;19)(p11;p11) and dic(19;22)(p11~p13;q11~q13). These findings provide evidence that chromosome behavior subsequent to telomeric instability plays a role in the formation of clonal deletions, dicentrics, and unbalanced translocations of 1p.

Spectral karyotyping (SKY) analysis for complex chromosomal abnormalities in adult non-endemic Burkitts lymphoma. *B.B. Wang¹, C.Y. Chung², C.S. Chang², L.J. Hsieh¹, H.M. Chang³.* 1) Ctr Medical Genetics, Changhua Christian Hosp, Changhua, Taiwan; 2) Division of Hemato-oncology, Department of Internal Medicine Changhua Christian Hosp, Changhua, Taiwan; 3) Department of Pathology Changhua Christian Hosp, Changhua, Taiwan.

Some non-Hodgkin lymphomas (NHL) have clonal chromosomal abnormalities with specific or non-specific changes. Such abnormalities are often difficult to identify by conventional G-banding, but relatively easy to refine by spectral karyotyping (SKY) analysis. SKY analysis is a new molecular cytogenetic method that is based on the hybridization of 24 fluorescently labeled painting probes to a chromosome spread, allowing the visualization of chromosomes in different colors in one experiment. We report a case of non-endemic Burkitts lymphoma with involvement of many chromosomal changes in a bone marrow specimen. By conventional G-banding, the analysis showed multiple numerical and structural chromosomal aberrations involving 2p, 3p, 6p, 8q, 9p, 14q, 18q and X chromosome, but with no further information about the breakpoints or interchromosomal changes. However, SKY analysis was able to refine all the aberrations, and even revealed further the presence of a hidden translocation between chromosomes 8 and 14, a frequently observed reciprocal translocation in Burkitts lymphomas. Thus, with combined use of G-banding and SKY analysis, we were able to refine all the complex chromosomal changes, confirmed the presence of a 8;14 translocation, and foremostly, redesignated the karyotype to be 51,XXX, t(2;9) (p23;p13), +der(3)t(3;18)(p25;q21), t(6;9)(q21;p22), t(8;14) (q24.1;q32),+12,+13, -18, +der(18) t(8;18) (q24.1;q32)x2.

Cytogenetic and FISH studies of five cases of solid tumor.. *C.W. Yu¹, Y. Yang¹, F. Shipkey², J. Keeton³, J. Wyatt-Ashmead².* 1) Dept. of Preventive Medicine; 2) Dept. of Pathology; 3) Dept. of Pediatrics, University of Mississippi Medical Center, Jackson.

Specific chromosomal anomalies are frequently associated with particular cancers. The analysis of chromosomes provides useful information for cancer classification and treatment. We report here the cytogenetic and FISH studies from two meningiomas, one ovarian and two renal tumors. **Case 1.** BG, a 56-year-old white female, was referred due to the gradual onset of decreased vision, headaches and overall slowing of mentation. CT scan found a large frontal meningioma. Cytogenetic and FISH studies of the tumor indicated a large pericentric inversion 7. **Case 2.** JT, a 44-year-old male, was referred because of moderate double vision and difficulty walking. CT scan revealed a left sphenoid wing meningioma. Cytogenetic study of the tumor revealed a 7/22 translocation and monosomy 22. **Case 3.** JP, an 18-year-old black female with a history of Wilm's tumor, was referred because of a sex-cord stomal tumor. CT scan revealed a pelvic mass that was found to be stage III-C ovarian cancer. Cytogenetic and FISH studies of the abdominal mass revealed a derivative chromosome 11 resulting from a deletion and a pericentric inversion. **Case 4.** LN, a 6-year-old black male was referred due to bowel pain. Renal ultrasound identified an echogenic mass on the anterior surface of the right kidney. Cytogenetic and FISH studies of the tumor revealed a derivative 15 consisting of chromosome 15 and the long arm of 7. **Case 5.** NS, a 4-year-old black male, was referred because of decreased activity. On evaluation, a left renal tumor was found. Cytogenetic study of the renal mass indicated a clonal evolution with chromosome number ranging from 53 to 55. Abnormality of chromosome 7, either structural or numerical, was seen in four of the five tumors that we studied. Structural rearrangement of chromosome 11 and either structural change of chromosome 22 or monosomy 22 were found in the ovarin tumor and the meningioma respectively.

TEL/ABL gene fusion shown by FISH using commercially available probes in a patient with CML, eosinophilia and a t(9;12)(q34;p13). *M.J. Pettenati*¹, *M. Beaty*², *Yi-K. Keung*³. 1) Depts Pediatrics/Medical Genetics; 2) Pathology; 3) Internal Medicine/Hematology and Oncology, Wake Forest Univ Sch Medicine, Winston-Salem, NC.

CML is a myeloproliferative disorder characterized by cytogenetic or molecular genetic evidence of a t(9;22). We identified a case of Ph- CML with eosinophilia and a t(9;12)(q34;p13) with a TEL/ABL gene fusion shown by commercially available FISH probes.

The individual presented with persistent leucocytosis after an episode of lobar pneumonia. The marrow was 100% cellular composed largely of neutrophilic and eosinophilic precursors and was pathologically consistent with CML. An initial RT-PCR analysis of BCR/ABL showed a positive gene rearrangement. Karyotypic analysis revealed a 46,XX,t(9;12)(q34;p13). FISH with BCR/ABL probes failed to detect any fusions. A repeat RT-PCR analysis failed to detect a bcr/abl fusion. A follow-up cytogenetic study was unchanged. There are 7 reported cases of TEL/ABL gene rearrangements by DNA analysis, one with a chromosome abnormality. By using commercially available FISH probes, LSI BCR/ABL and TEL/AML1, we were able to demonstrate fusion of the ABL and TEL at 9q34.

The TEL(ETV6) gene is a member of the E26 transformation-specific family of transcription factors located at 12p13. Its role of leukemogenesis has been shown as a fusion partner in cases of t(5;12)(q33;p13), t(12;22)(p13;q11) [MN1/ETV6], t(3;12)(q26;p13) [ETV6/EVI1], and t(12;21)(p13;q22) [ETV6/AML1]. TEL(ETV6) is a very promiscuous gene involved in with over 40 different chromosome rearrangements. TEL is the only non-BCR fusion partner for ABL in human leukemia reported to date. Cytogenetically, this 9;12 chromosomal translocation can be difficult to detect. One can demonstrate the fusion of ABL and TEL using commercially available FISH probes in combination. This combination of probes may prove useful in the cytogenetic evaluation of cases with a similar cytogenetic abnormality or CML cases lacking the 9;22 translocation/fusion.

Gain of Chromosomes 7/7q and 11p in neuroblastoma. *R.L. Stallings^{1,2}, M. Mullarkey¹, C. Breen¹, J. Howard¹, A. Dunlop¹, M. McDermott³, F. Breatnach³, A. O'Meara³.* 1) National Ctr Medical Genetics, Our Lady's Hosp Sick Children, Dublin, Ireland; 2) Faculty of Medicine, University College Dublin, Dublin Ireland; 3) Our Lady's Hospital for Sick Children, Dublin, Ireland.

Loss of chromosome 11q material is a common genetic abnormality of advanced stage neuroblastoma and represents a distinct genetic subtype of tumour. This abnormality is frequently accompanied by unbalanced gain of the 17q region (>90%) and loss of chromosome 3p material (~70%). Gain of 11p material has also been reported in neuroblastoma with 11q loss, but at a considerably lower frequency (~10%) than the losses and gains for the 3p and 17q regions. Results reported here, however, indicate that gain of 11p may occur more frequently in the 11q- neuroblastoma subtype than was previously realized. CGH analyses of neuroblastoma from eleven patients indicated that six out of eleven tumors (55%) with loss of 11q also possessed gain of chromosome 11p. The shortest region of 11p gain was 11p11.2 to p14. G banding and FISH analysis performed on tumor cells from primary and metastatic sites indicates that gain of the 11p region occurs secondary to an unbalanced t(11;17) that led to 11q loss (and 17q gain). Gain of chromosome 7 (17/43 tumours) or 7q (5/43 tumours) material is a very common abnormality in neuroblastoma, but unlike gain of 11p, it is not restricted to the 11q- subtype and is prevalent in both low and high stage tumours. The significance of chromosome 7 gain is underscored by the fact that it was the sole abnormality detected in one tumour. We conclude that gain of 7/7q and 11p material may contribute to either neuroblastoma tumorigenesis or progression.

Comparison of cancer cell lines by karyotype and comparative genomic hybridization. *S.L. Wenger¹, J.R. Senft², L.M. Sargent², S.G. Grant³*. 1) Dept Pathology, West Virginia Univ, Morgantown, WV; 2) NIOSH, Genetic Susceptibility Lab, Morgantown, WV; 3) University of Pittsburgh, Dept Environmental and Occupational Health, Pittsburgh, PA.

Two cancer cell lines, MCF7 and ISHIKAWA, were each obtained from two sources, which maintained cells in different culture media. MCF7 was derived from breast tumor: #1 was obtained from ATCC at passage 149 and passaged an additional 16 times; #2 was cultured in another laboratory for unknown passages and subcultured an additional 95 times. Cell line #2 had a baseline HPRT mutation frequency 10-fold higher than #1. ISHIKAWA was derived from an endometrial tumor: #3 was obtained from European Collection of Cell Cultures passaged more than 3 times, was ER positive, and subcultured 130 more times; #4 was subcultured unknown times in another laboratory, was ER negative, and subcultured an additional 132 times. Cell line #4 had a baseline HPRT mutation frequency 1400-fold higher than #3. MCF7 cultures had complex karyotypes, however, similarities included up to 83 chromosomes, additional chromosomes 2,3,4,5,6,7,8,9,10,12,13,14,15,17,19,20, structural abnormalities add(3)(p25),del(6)(q21),add(22)(q13), and at least 1 of up to 17 marker chromosomes in common. The ISHIKAWA cultures each had up to 60 chromosomes with 4-6 markers, but only a missing X in common. CGH studies were performed using different colored fluorochromes to label each of the two MCF7 or ISHIKAWA cell lines, which were then co-hybridized to normal metaphases. Differences seen between the pairs of MCF7 and ISHIKAWA cultures reflect karyotype differences. Some initial DNA polymorphic data for the ISHIKAWA cell lines (Dr. R. Bamezai, New Delhi, India) suggest that they may not be derived from the same established cell line. Our studies demonstrate the utilization of CGH for comparing cell lines originating from the same specimen, but undergoing karyotypic and mutation rate changes due to different culture conditions and passage numbers.

Is a 20-cell cytogenetic analysis necessary for neoplastic bone marrow specimens? *B. Roland*^{1,2}. 1) Dept Pathology & Lab Medicine, University of Calgary, Calgary, AB, Canada; 2) Calgary Laboratory Services.

Bone marrow specimens in neoplastic disorders sometimes have a minority of cells with a chromosome abnormality. In order to detect small abnormal clones, North American guidelines require analysis of 20 or more cells from bone marrow and neoplastic blood specimens when all cells are normal. The goal of this study is to determine the effect of analysing fewer than 20 cells on the rate of detection of abnormalities.

Records from 4081 bone marrow specimens that had been analysed in one laboratory between 1991 and 2001 were examined retrospectively. At diagnosis, 20 cells had been analysed routinely; at follow-up, 30 cells had been examined for previously-identified abnormalities. 890 samples had an abnormal karyotype, and 479 of those were mosaic, with both normal and abnormal cells present. For each mosaic abnormal specimen, the following data were recorded: referring and final diagnosis, new diagnosis vs. treated, % abnormal cells, the cell at which an abnormality was first detected, and the cell at which the abnormality was confirmed as clonal.

Of the 479 mosaic abnormalities, 6 were first detected after cell #15. Therefore 6/479 (1.2%) of mosaic abnormalities, or 6/890 (0.7%) of all abnormalities that were detected by a 20-cell analysis would have been missed by a 15-cell analysis. Subgroups of data were also analysed, with similar results for samples at diagnosis, after treatment, and for most referring diagnoses and final diagnoses.

In conclusion, a 15-cell analysis of bone marrow specimens will detect 98.8% of the mosaic abnormalities and 99.3% of all abnormalities that are detected by a 20-cell analysis. These data should be considered when reviewing guidelines for the extent of cytogenetic analysis of bone marrow specimens. For some specimens at the time of diagnosis, a 15-cell analysis may be adequate, if additional cells are checked to confirm the clonality of any single-cell abnormalities that are detected.

Chromosomal imbalances and RET rearrangements in radiation-induced thyroid tumors. *H.E. Richter¹, A. Walch², J. Smida², L. Hieber¹, E. Lengfelder³, E.P. Demidchik⁴, H.-U. Weier⁵, H. Zitzelsberger¹.* 1) GSF-National Research Center for Environment and Health, Institute of Molecular Radiobiology, Neuherberg, Germany; 2) Institute of Pathology, Technische Universitaet Muenchen, Germany; 3) Radiobiological Institute, University of Munich, Germany; 4) Center for Thyroid Tumours, Minsk, Belarus; 5) Department of Subcellular Structures, Life Sciences Division, University of California, E.O. Lawrence Berkeley National Laboratory, Berkeley, CA, USA.

The aim of this study was to investigate cytogenetic changes in radiation-induced papillary thyroid tumors from children exposed to the radioactive fallout following the Chernobyl nuclear accident in 1986. The control group was comprised of tumors without radiation history. Methods used were comparative genomic hybridization (CGH) and RT-PCR analysis for RET expression. Results of this study were expected to provide leads to molecular cytogenetic mechanisms involved in radio-carcinogenesis. Frozen and micro-dissected paraffin-embedded tissues were available for CGH analyses. Ligation-mediated PCR was performed for amplification of whole genomic tumor DNA. Additionally, the specimen RNAs were analyzed by RT-PCR for specific rearrangements of the RET proto-oncogene (RET/PTC1, RET/PTC3) and general expression of the RET tyrosine kinase domain. For cases with insufficient RT-PCR amplification Southern blot analysis of PCR products were performed with radioactive labeled oligonucleotide probes. CGH analysis showed chromosomal imbalances in 23% (8 of 35) of the childhood tumors involving mostly chromosomes 1, 2, 5, 19, 20 and 22. In the non-irradiated control group from Munich, we detected copy number changes in 5/8 cases. At present specific RET rearrangements or expression of the RET domain was found in 9 of 16 childhood and 1 of 3 of the control tumors. Only one radiation-induced thyroid tumor with a rearranged form of the RET proto-oncogene (PTC1) showed additionally chromosomal imbalances detected by CGH suggesting an involvement of additional genes in the carcinogenic process. The authors gratefully acknowledge support from the US NIH (1R01 CA80792, 1R33 CA88258).

Multiple factors influence aneuploidy-driven chromosomal instability in oral squamous cell carcinoma. S.

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Oral squamous cell carcinoma (OSCC) arises through a complex, multistep process of genetic alterations. Previous studies have shown that OSCC cells exhibit near-triploid karyotypes and contain various clonal structural and numerical abnormalities. We hypothesized that in OSCC cells, chromosomal instability (CIN) is influenced by the state of aneuploidy within a cell. To test this hypothesis, we isolated six clones from two of our OSCC cell lines. Using a panel of centromere probes specific for chromosomes 4, 6, 7, 9, 11, 17, 20, and X, fluorescence *in situ* hybridization (FISH) was carried out on each of the clones. Variations in chromosome number both within clones and between clones of the same cell line were observed. However, despite similar chromosomal copy number, 'homologs' of a chromosome were structurally distinct from cell to cell ("marker chromosome evolution"). Spectral karyotyping (SKY) analysis revealed the segmental origin of these structurally aberrant chromosomes in cells within a clone. Our findings suggest that the aneuploidy observed in OSCC cells may be the result of both intrinsic chromosomal factors and extrinsic cytoskeletal factors, and that CIN is driven by their combined effects.

B-cell prolymphocytic leukemia with translocation 11;14: is it B-PLL or is it a leukemic variant of mantle cell lymphoma? C.A. Tirado, V. Golembiewski-Ruiz, J.O. Moore, P.J. Buckley, B.K. Goodman. Depts of Pathology and Medicine, Duke University Medical Center, Durham, NC.

B-cell prolymphocytic leukemia (B-PLL) is a very rare disease affecting the blood, bone marrow and spleen. It is characterized by a lymphocytic count greater than 100,000 consisting of medium to large round lymphoid cells with prominent nucleoli (prolymphocytes), marked splenomegaly, and median age of 70. Our patient presented with a fairly rapid onset of symptoms including weight loss, night sweats and dysnea or exertion. His WBC was 200,000 with 50% lymphocytes. He had massive splenomegaly and was referred to our institution for further evaluation. His white count continued to climb. Bone marrow and peripheral blood samples were sent for pathologic and cytogenetic evaluation. He had a B-cell neoplasm consisting of greater than 55% of peripheral blood cells, with prominent nucleoli, consistent with prolymphocytes. The cells expressed monoclonal surface immunoglobulin, were moderately CD5+, dimly FMC7+ and CD23 negative. Chromosome analysis showed 40-43 chromosomes with a complex karyotype including translocation 11;14, loss of chromosomes 3, 8 and 13 and iso 17q. FISH analysis showed the CCND1/IGH rearrangement with a variant pattern indicating either a breakpoint upstream of the CYCLIN D1 major translocation cluster or deletion of sequences upstream of this region. These results pose the question whether to consider this case as a B-PLL mimicking mantle cell leukemia/lymphoma or a mantle cell lymphoma (MCL) with an atypical blastoid presentation. About 1/3 of B-PLL cases and most MCL cases are CD5 positive. Both B-PLL and MCL are CD23-, FMC7+ and express IgM. While 75% of MCL are 11;14 positive, the translocation is seen in only about 20% of B-PLL cases. Deletions of chromosome 13 and the TP53 locus at 17p have been reported in both entities. Prognosis and treatment differ for these two disorders. It is critically important to fully characterize complex cases of hematologic malignancy such as this one, that share specific cytogenetic or molecular rearrangements, in order to generate criteria that will significantly affect clinical prognosis and treatment options.

The use of sub-telomere FISH and paint probes to clarify the origin of derivative chromosomes in bone marrow studies. *J.L. Smith, J.P. Cook.* Dynagene, Houston, TX.

Cytogenetic interpretation of bone marrow studies may be complicated by the presence of derivative or marker chromosomes whose identity cannot be conclusively determined by G banding. The ideal methodology for determining the exact composition of such chromosomes is by 24 color FISH; however, not every laboratory has access to this technology. We present two patients on whose samples paint probes and/or telomere FISH were useful in clarifying the composition of a derivative chromosome. Patient 1 was a 79 year old male referred for pancytopenia. The stemline and sideline had a supernumerary derivative chromosome which was very similar in appearance to the t(1;19)(q23;p13) commonly observed in lymphocytic disease. Due to the patient's age and recent reports of a t(1;19)(p13;p13.1) in myeloid disease, FISH analysis was performed. Telomere FISH (Vysis) showed one end of the derivative chromosome to hybridize with 1q and the other end to hybridize with 19p, supporting the probability that this was the 1;19 translocation consistent with myeloid disease. Since there was some question as to whether the derivative chromosome arose through centric fusion of 1q and 19p, the CEP1 a satellite probe was also hybridized to metaphase cells; both number 1 chromosomes and the derivative chromosome had CEP 1 signals. FISH analysis using paint probes for 1 and 19 from the OctoChrome panel (Cytocell) showed most of the derivative to paint with chromosome 1 and a small portion of one end to paint with 19, further supporting the interpretation of a supernumerary der(1)t(1;19) (p13;p13.1). Patient 2 was a 46 year old male referred for anemia; his condition was further complicated by a lung mass. He had a very complex karyotype which included a chromosome originally designated as der(1;19)(q10;p10). However, the derivative chromosome hybridized only with 1p and 1q subtelomeric probes indicating a deletion of 1p not a translocation involving 19p. In both patients, the clarification with telomere probes (and paint probes in one patient) allowed more accurate interpretations of the cytogenetic abnormalities which led to more accurate clinical significance statements.

Telomeric Association between Chromosomes 14 and 19 at bands p13 and q13.4 in a case of MDS/AML. *K.H. Ramesh, V.R. Pulijaal, C.T. Harrison, T. Zhou, S.K. Gogineni, D.S. Xu, L.A. Cannizzaro.* Dept Pathology, Montefiore Medical Ctr/AECOM, Bronx, NY 10461.

A 91 year old caucasian male with a 10 year history of well-differentiated adenocarcinoma of the prostate, now presenting with pancytopenia was referred for unstimulated peripheral blood cytogenetic analysis to rule out Myelodysplastic syndrome (MDS)/Myeloproliferative disease (MPD). Bone marrow biopsy showed a hypercellular marrow with left shifted granulopoiesis, 1-2 % myeloblasts and diffuse increase in reticulin fiber. Immunohistochemical studies for cytokeratin and prostate specific antigen were negative. Flow cytometry was not performed. Based on these findings the patient was diagnosed with secondary refractory anemia. The patient was put on supportive treatment and subsequently died. Cytogenetic analysis revealed a 45,XY,del(2)(q23q33),del(5)(q13q33),-7,tas(14;19)(p13;q13.4) karyotype in 35 % of the cells with the rest being normal. The abnormal clone showed loss of chromosome 7, and the structural anomalies included interstitial deletions of chromosomes 2 and 5 at bands q23q33 and q13q33 respectively, and telomere association (tas) between chromosomes 14 and 19 at bands p13 and q13.4. Fluorescence in-situ hybridization (FISH) analysis with painting probes for chromosomes 2, 5, 14 and 19, and telomere probes for 2q, 5q and 19q was performed to confirm the cytogenetic findings. A diagnosis of MDS evolving to AML was suggested based on the findings of deletion 5q and monosomy 7 and the secondary changes which included tas(14;19)(p13;q13.4). The occurrence of clonal tas in MDS/AML is a rare cytogenetic phenomenon in which there is fusion of terminal ends resulting in unstable dicentric, multicentric and ring chromosomes. Chromosomes 14 and 19p are frequently involved in tas. These are considered to be secondary changes when they occur with other characteristic anomalies that are frequently associated with MDS/AML. The identification and characterization of specific chromosomal anomalies is critical, especially when a hematological diagnosis is pending or inconclusive. This has important implications in the classification, treatment and prognosis of MDS/AML.

Chromosomal imbalances in flat and exophytic lesions of the human colon. *H. Zitzelsberger¹, H.E. Richter¹, A. Walch², P. Slezak³, M. Werner².* 1) GSF-National Research Center for Environment and Health, Institute of Molecular Radiobiology, Neuherberg, Germany; 2) Institute of Pathology, Technische Universitaet Muenchen, Germany; 3) Endoscopy Unit, Department of Gastroenterology, Karolinska Hospital, Stockholm, Sweden.

The aim of the study is to compare CGH results from flat and exophytic lesions of the human colon to study whether there are different avenues for colorectal cancer formation. Microdissected paraffin-embedded tissues were used for CGH analysis of histologically defined areas of colorectal lesions. Ligation-mediated PCR was performed for amplification of whole genomic tumour DNA. No aberrations were detected in normal epithelium (n=9). CGH analysis showed chromosomal imbalances in 71% (22 of 31 cases) of flat lesions and in 46% (13 of 28 cases) of exophytic lesions. In flat adenomas, DNA losses are frequently detected on chromosomes 16p, 17p, 20q and DNA gain is frequent on chromosome 8q. In carcinomas, gains on 8q, 20q as well as losses on 8p, 17p and 18 are recurrently observed. Exophytic adenomas showed preferentially DNA losses and gains of whole chromosomes indicating aneuploidy in these tumors. Distinct differences in chromosomal aberrations are detected in flat and exophytic lesions of the human colon. This indicates that there might be more than one avenue for colorectal cancer formation which is in accordance with morphological characteristics of polypoid cancers and cancers arising de novo, i.e. without any adenomatous component.

The Relationship between TOP2A Gene Copy Number and Topoisomerase IIa Protein Levels in Human Breast Tumors. *R.E. MUELLER¹, R. PARKES¹, F.P. O'MALLEY^{2,3}*. 1) Samuel Lunenfeld Research Inst, Toronto, Ontario, Canada; 2) Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto; 3) Department of Laboratory Medicine and Pathobiology, University of Toronto.

Background: Clinical studies have suggested that tumors with HER2 gene amplification are particularly sensitive to anthracycline drugs used in adjuvant breast cancer therapy. The TOP2A gene, located adjacent to the HER2 gene on human chromosome 17q21-22, encodes topoisomerase IIa and is frequently altered in conjunction with HER2. Topoisomerase IIa is a direct molecular target for anthracycline drug action. Changes in the TOP2A gene copy number may result in altered levels of topoisomerase IIa in breast tumor cells and could contribute to differences in patient response to anthracycline therapy. **Design:** We evaluated a group of 43 formalin fixed, paraffin embedded, invasive breast tumor samples for topoisomerase IIa protein expression and cell proliferation using immunohistochemical staining, and for the copy number of TOP2A, HER2 and chromosome 17 (where both these genes normally reside) using fluorescence in situ hybridization. **Results:** Thirty-two samples had HER2 gene amplification. Of these, 11 samples (34.4%) had amplification of both genes while 16(50%) had a normal TOP2A gene copy number. Five samples (15.6%) were amplified for HER2 but showed deletion of TOP2A relative to the number of copies of chromosome 17. One sample had deletion of both HER2 and TOP2A genes. Ten tumor samples had no amplification of either gene. There was no evidence of a difference in the mean topoisomerase IIa protein level for TOP2A amplified (23.4%) vs unamplified (22.4%) cases ($p=.80$). **Conclusions:** Unlike HER2 where gene amplification is tightly linked to protein overexpression, no association was seen between the amplification of the TOP2A gene and protein expression levels of topoisomerase IIa in breast tumor cells. Protein levels of topoisomerase IIa determined by immunohistochemistry were not correlated with gene copy number in these samples suggesting that the analysis of TOP2A gene amplification in breast tumors will not identify all patients with high expression levels of topoisomerase IIa.

Evaluation of mouse telomere length with optimized Primed In Situ (PRINS) labeling technique. *J. Lavoie¹, M. Bronsard¹, M. Lebel², R. Drouin¹.* 1) Unite de recherche en genetique humaine et moleculaire, Universite Laval, Hop St-Francois d'Assise, CHUQ, Quebec, PQ, Canada; 2) Centre de recherche en Cancerologie, Universite Laval, Hop Hotel-Dieu de Quebec, CHUQ, Quebec, PQ, Canada.

Telomeres are chromosomal elements composed of (TTAGGG) DNA repeats. Telomeres are required for genomic stability; they protect chromosomes from degradation or end-to-end fusion, and are implicated in regulating the replication and senescence of cells. The gradual loss of telomeric DNA repeats has been linked to cellular aging and tumorigenesis. It is now thought that the shortening of specific telomeres at certain chromosome ends may be critical to cellular function. Therefore, methods that allow the estimation of the length of specific telomeres are of considerable interest. Individual telomeres can be analyzed using one of two methods: (i) the quantitative fluorescence in situ hybridization (Q-FISH) method, which uses the peptide nucleic acid probe and is the most popular, and (ii) the primed in situ (PRINS) labeling reaction, which produces a labeled copy of the telomeric DNA repeats in situ. We tested different conditions for DNA denaturation, labeling temperature, and other variables to optimize the PRINS reaction for detection and quantification of mouse telomeric DNA repeats. The specificity, efficiency and uniformity of staining were evaluated using digital fluorescence microscopy. Uniform staining is of paramount importance if PRINS is to provide telomere fluorescence intensity values that are sufficiently accurate for measurement of telomere length. Staining uniformity is measured by comparing the values of sister chromatids, as they are expected to contain essentially the same number of telomeric DNA repeats. Optimization of the labeling reaction resulted in an average detection efficiency of 99% and a good correlation between the values derived from sister chromatids was observed. We conclude that PRINS is a reliable method for telomere length evaluation and, compared to FISH, presents some advantages including a higher cost efficiency and less technical time. These advantages may encourage wider use of the PRINS technique for detection and quantification of telomeres in situ.

***In vitro* immediate and delayed cytogenotoxic effects of environmental concentrations of cadmium(II) in human lymphocytes.** M. Cojocaru^{1,3}, F. Fortin¹, S. Chakrabarti², N. Lemieux^{1,3}. 1) Dept. Pathologie et Biologie Cellulaire, Université de Montréal; 2) Dept. Travail et Hygiène du milieu, Université de Montréal; 3) Centre de recherche, Hôpital Sainte-Justine, Montréal, Qc, Canada.

Cadmium is a known carcinogenic metal, but its mechanism of action and the range of doses at risk for humans are not clarified. Our purpose is to study the immediate and delayed cytogenotoxicity of environmental concentrations of cadmium chloride (CdCl₂) in human lymphocytes, after an acute exposure (2hrs). The techniques used were the electron microscopy *in situ* end-labeling (EM-ISEL) and the sister-chromatid exchanges (SCE). We also investigated the induction of apoptosis and the effect on the cell cycle kinetics. The viability study showed that initially non-cytotoxic high concentrations (>150µM) have a delayed cytotoxicity, manifested after a 24 hour recovery. The cytogenotoxicity study revealed that concentrations equal or superior (0,01; 0,06; 0,1 and 1µM) to the cadmium blood concentration in smokers have a significant clastogenic effect, when measured immediately after exposure. On the other hand, the lowest tested concentration (0,002µM), equivalent to the cadmium plasma level in non-smokers, seems to act in a phase-dependent manner, producing a significant clastogenic effect only in interphase chromatin but not in chromosomes. Furthermore, a non-threshold delayed clastogenic effect was observed: while certain concentrations (0,002; 0,06 and 0,1µM) allow the recovery of the DNA integrity, other concentrations (0,01 and 1µM) significantly increase the frequency of DNA single-strand breaks at a delay of 48 hours after exposure. This last effect is associated with a delayed induction of apoptosis only in the case of the 0,01µM dose. Contrary to EM-ISEL, the SCE test has lead to negative results even at the highest doses. On the other hand, a significant slowing down in the cell cycle kinetics, dependent on the dose and on the delay after exposure, was observed. In conclusion, cadmium is a clastogenic agent at environmental concentrations. Furthermore, our study has provided more relevant information on its genotoxicity and on the related outcome by showing the persistence of induced lesions.

Inactivation of Rrb1p, a yeast nucleolar protein involved in ribosome biogenesis, results in chromosomal instability. A. Killian¹, N. Le Meur¹, P. Hieter², J-M. Flaman¹, T. Frebourg¹. 1) INSERM EMI 9906, Faculty of Medicine, 76183 Rouen, France; 2) Center for Molecular Medicine and Therapeutics, University of British Columbia, Vancouver, Canada.

Recent data have indicated that alteration of the spindle checkpoint, which controls the correct attachment of chromosomes to the spindle, might be involved in cancer. SAGE (Serial Analysis of Gene Expression) has previously been used to determine the yeast transcriptome in response to the activation of the spindle checkpoint induced by nocodazole. We have initiated functional analysis of genes induced by the activation of the spindle checkpoint and identified by SAGE. One of the genes, *ymr131c/RRB1*, is an essential gene encoding a nucleolar WD repeat protein which has recently been shown to bind the ribosomal protein rpl3 and to be involved in the assembly of the 60S ribosomal subunit. We have generated a temperature sensitive (ts) *RRB1* mutant yeast strain in which chromosomal instability was detected by a sectoring phenotype. The ts *RRB1* mutant strain demonstrated at semi-permissive temperature an abnormal sensitivity to benomyl suggesting that mutation of *RRB1* alters the spindle checkpoint. Screening of a yeast genomic library in the ts *RRB1* mutant strain led us to identify a genetic interaction between *RRB1* and *RPL3*. This study shows that inactivation of a protein involved in ribosome biogenesis may alter chromosomal segregation through an alteration of the spindle checkpoint.

Cancer incidence in first-degree relatives of a population-based set of cases of early-onset breast cancer with a known BRCA1 and BRCA2 mutation status. *H.L. Olsson, N. Loman, A. Bladstrom, O. Johannsson, A. Borg.* Dept Oncology, Univ Hosp, Lund, Sweden.

We have studied relative and cumulative cancer incidence in relatives of a population-based set of early onset breast cancer patients (N=203) with a known BRCA1 and BRCA2 germline mutation status. Among first-degree relatives (FDRs) of BRCA1 and BRCA2-mutation carriers (N=79), the standardized incidence ratio of breast cancer SIR=8.0 (95% CI=3.4-15.7) and ovarian cancer SIR=14.7 (95% CI=3.0-43.0) were elevated. In FDRs of women without a BRCA1 or BRCA2 mutation (N=705) the incidence of breast cancer was increased SIR=2.3 (95% CI=1.6-3.3). In addition, SIR of prostate cancer was elevated SIR=1.6 (95% CI=0.97-2.6). In a subgroup of women with at least two FDRs with breast cancer, but without an identified BRCA1 or BRCA2 mutation in the family (N=180), incidence of breast cancer was increased SIR=5.5 (95% CI 3.0-9.2). Cumulative incidence of breast cancer up to age 50 and 70 were calculated for FDRs of index-women with a BRCA1 or BRCA2 mutation: 19.1% (95% CI 8.8-41.8%) and 35.3% (95% CI 17.5-62.8%) respectively, translating to 36.8% (95% CI 21.0-52.6%;) and 65.3% (95% CI 42.2-88.4%) risks in BRCA1 or BRCA2 mutation carriers; for FDRs of index individuals without an identified BRCA-mutation: 3.6% (95% CI 1.3-5.7%;) and 12.8% (95% CI 6.5-15.7%); and finally for a subgroup of FDRs of index individuals without a BRCA1 or BRCA2 mutation but with at least one additional first- or second degree relative with breast cancer: 8.4% (95% CI 3.1-16.6%) and 27.7% (95% CI 13.4-46.2%) at ages 50 and 70. In FDRs of index-individuals with breast cancer <36 years of age without BRCA1 or BRCA2 mutations breast cancer risk is increased three-fold; if the index-individual was 36-41 the risk was only doubled. In addition to breast cancer, the incidence of prostate cancer is elevated among individuals with a FDR with breast cancer before age 36 but no BRCA1 or BRCA2 mutation in the family.

HY558, a novel cell cycle inhibitor, induces G1/G2 arrest and apoptosis in the human cancer cells. *C. Lee, H. Lim, M. Kim, Y. Cho.* Department of Medical Genetics, Hanyang University, Seoul, Seoul, Korea.

Proliferative disorders, cancer, are recognized as diseases of the cell cycle. In the course of screening for a novel cell cycle inhibitor, a novel CDK 1 inhibitor, HY558, was found from *Penicillium minioluteum* F558. This study reports on the biological properties of HY558 as a cell cycle inhibitor and apoptosis inducer in cancer cells. Inhibition of cancer cell proliferation: The antiproliferative effects of HY558 were assessed in six human cancer cell lines and normal lymphocytes as the control. The growth of all of the cancer cells tested, except for A549 and the normal lymphocyte cell, was inhibited in a dose-dependent manner by HY558. The estimated IC₅₀ in these cancer cell lines ranged from 0.25 to 0.33 mM. Interestingly, HY558 demonstrated no antiproliferative effect on the normal lymphocyte cells, and a low level of inhibition on the A549. Inhibition of cell cycle regulation: Flow cytometric analysis in HepG2 cells revealed an appreciable arrest of cells in the G1 and G2/M phase after treatment with 0.46 mM of HY558. Furthermore, HY558 has little effect on the expression levels of CDK 4/6, cyclin D1, and E, but decreases the levels of CDK2, and CDK1, as well as cyclin A/B1 in the HeLa cells, in which only G2/M phase arrest has been detected in flow cytometric analysis. Induction of apoptosis: To investigate the apoptotic induction by HY558, the DNA fragmentation of HeLa cells due to apoptosis was measured by a TUNEL assay. When HeLa cells were incubated with 0.46 mM of HY558 for 24 h, an apoptotic DNA fragmentation was observed. To understand the mechanism of HY558-induced apoptosis, we examined the change of the level of intracellular proteins related to apoptosis, such as Fas-L, Fas, Bcl-2, Bax, caspase-8 and caspase-9 in the HeLa cells. HY558 induced up-regulation of Bax, and activation of pro-caspase-9. However, there were no significant changes in expression of Fas, Fas-L, and Bcl-2. Furthermore, HY558 induced proteolytic cleavage of inactive pro-caspase-3 into its active form, and subsequent cleavage of its substrate PARP. The activation of caspase-9 in the HeLa cells is likely to occur via the mitochondria pathway.

Incorporating cytochrome P450 3A4 genotype expression and FT-IR/ Raman spectroscopy data as means of identification of breast tumors. *S.O. Miller¹, G.P. Gideon¹, C.M. Howard³, H. Tachikawa³, S.A. Bigler², W.H. Barber¹, M. Angel¹, D.O. McDaniel¹.* 1) Surgery, Univ of MS Medical Ctr, Jackson, MS; 2) Dept Medicine, Univ of MS Medical Ctr, Jackson, MS; 3) Depts Biology and Chemistry, JSU, Jackson, MS.

This study first seeks to determine importance of cytochrome P450s (CYPs) in breast cancer and second it utilizes the application of Fourier transform-infrared (FT-IR) and Raman spectra as a diagnostic tool in evaluating breast tissues. Making a diagnostic decision in early stage of breast cancer relies in part on biological presentation of breast tissue components. CYPs are involved in the production of potentially carcinogenic estrogen metabolites and the activation of environmental carcinogens and organic solvents. Such contaminants have been associated in the developmental pathways of mammary tumorigenesis. Thus in parallel studies we designed experiments to compare by RT-PCR, the CYPs 1A2, 1B1, 2E1 and 3A4 mRNA transcript levels and by FT-IR the relative level of carotenoids, lipids and heme-containing components with the pathology of the breast tissues. The CYP 1A2 and 2E1 mRNA transcripts were expressed in normal breast and tumor tissues. The mRNA CYP1B1 was in the order of 10-fold higher in some tumor tissues as compared with normals, but it was also detected in one breast reduction sample, with normal pathology. The CYP 3A4 mRNA transcript (380-bp fragment) was increased in the order of 10-fold in breast tumor as compared with normal tissues. In addition a 260-bp truncated fragment of 3A4 mRNA was detected in normal and reduction samples particularly in samples that the larger fragment was absent. Raman spectra of breast tumor tissue were demonstrated by an increase of heme-protein related peaks as evidenced by the peaks at 341, 674 and 1360cm⁻¹. The appearance of the heme band may imply a high level of red blood cells due to a vasculature where tumor is developing. The spectra for lipid were remarkably decreased in the same region. In summary, a combined genotype analysis and Raman spectra approach could have an ideal diagnostic value in decision making during the early stage of breast cancer.

Program Nr: 333 from 2002 ASHG Annual Meeting

A Mathematical Programming Approach for Gene Selection and Tumor Classification. *M. Sun*¹, *M. Xiong*². 1) Dept Business, Univ Texas, San Antonio, San Antonio, TX; 2) Human Genetics Center, School of Public Health, University of Texas.

A mathematical programming approach is proposed for gene selection and tissue classification using gene expression profiles. A new mixed integer programming model is formulated for this purpose. The objective is to use a minimum number of genes to classify normal and tumor tissue samples as accurately as possible. Very good results were obtained for two data sets from the literature. These results show that the mathematical approach can rival or outperform more traditional classification methods.

Computational screening and experimental validation of tumor related alternative splicing in human genome. Z. wang, S. Lo, H. Yang, S. Gere, Y. Hu, K. Buetow, M. Lee. Laboratory of Population Genetics, National Cancer Institute, Gaithersburg, MD 20877.

Alternative RNA splicing plays an important role in the normal development of eukaryotic organisms and its abnormal regulation has been frequently associated with human diseases and cancers. The purposes of this research are: 1) to develop a new algorithm to identify as many splicing variants as possible; (2) to identify the alternative splicing sites that are unique to tumor; (3) to validate the tumor related RNA splicing by experiments. We used RefSeq NM as a standard splicing model, and 11,014 NM sequences were compared with 3,471,822 EST sequences by BLAST analysis. A total of 21,345 alternative splicing sites were detected genome-wide. We mapped EST to each of the splicing site and identified 4,932 alternative splicing sites that were unique to tumors. A list of all these potentially tumor related alternative splicing sites were available at: http://ncicblion.nci.nih.gov:8080leelab/jsp/as_info/AS_Tumor.html. To validate the computation result, RT-PCR experiments were performed to evaluate aberrant RNA splicing in four tumor tissues (breast, liver, lung and prostate) as well as their matched normal tissues. 65% of predicted aberrant splicing products were verified in at least one of four tissues. Our results showed that some aberrant splicing sites were tissue specific. We also found that some aberrant RNA splicing products were absent in normal tissue but were present in various of tumor tissues. Such aberrant RNA splicing products can potentially serve as valuable molecular markers for tumor diagnosis. A web enabled alternative splicing database was created for public querying by using key words such as gene symbol, locus link ID, UniGene ID or accession number (NM_*), and is available at: http://ncicblion.nci.nih.gov:8080/leelab/jsp/as_info/LPG_ASDB.jsp.

Genetic fingerprinting of patients after allogeneic bone marrow transplantation using recipient mouthwash samples. *M. Shahrooei*¹, *A. Aleyasin*¹, *K. Alimoghadam*², *A. Ghavamzadeh*². 1) National Research Center for Genetic Engineering and Biotechnology, Tehran, Iran; 2) Cancer and Hematology Research Center, Shariati Hospital, Tehran, Iran.

Serial monitoring of chimerism after allogeneic hematopoietic stem cell transplantation (HSCT) can be performed rapidly using PCR-based assays analyzing informative tandem repeat genetic markers. Prerequisite for this type of analysis is knowledge of donor and recipient pretransplantation genotypes. In some cases, recipient cells prior to BMT are not available for genotyping of recipient's VNTRs before transplantation. Our study was to evaluate the feasibility of mouthwash samples that contains mouth epithelial cells as BM recipients only after their genotype in blood cells. Of 19 patients who had undergone BMT, DNA was isolated from mouthwash cell pellets obtained from mouthwashes. PCR analysis of six STR loci on six chromosomes was performed. Even though the mouthwash cell pellets contained about 75% epithelial cells (presumably of recipient origin) and only about 25% leukocytes (presumably of donor origin), three of nineteen patients showed recipient genotype and the rest exhibited chimeric DNA patterns from 5.0% to 60.0%. It means that this DNA contained donor and recipient material in different ranges. From our results it appears that blood cells serve as preferential DNA source in mouthwash samples which can be obtain before BMT.

Implication of 9p deletion in 1p/19q-deleted oligodendrogliomas. *C. Godfraind¹, E. Rousseau^{1,2}, M.M. Ruchoux³, F. Scaravilli⁴, M. Vikkula².* 1) Division of Neuropathology, Université catholique de Louvain, Brussels, Belgium; 2) Laboratory of Human Molecular Genetics, Christian de Duve Institute of Cellular Pathology and Université catholique de Louvain, Brussels, Belgium; 3) Department of Neuropathology, Hôpital R. Salengro, Lille, France; 4) Institute of Neurology, London, UK.

Oligodendroglioma is a tumour originating from oligodendrocytes, the myelin forming cells in the central nervous system. This glioma preferentially occurs in adults. It is mostly located in cerebral hemispheres with a predilection to the frontal lobe. This lesion accounts for 5-33% of all gliomas. The wide range reported for tumour occurrence reflects inter-observer discordance in histological diagnosis. Genetic analysis of oligodendroglioma has associated 1p/19q-deletions to chemosensitivity. Recently, we and others have linked a specific histological definition to this subgroup of tumors, allowing the diagnosis to be made on histological criteria prior to any genetic analysis. Now, we have studied 9p deletions, p14, p15 and p16 methylation as well as p14 and p16 mutations in a series of 21 1p/19q-deleted oligodendrogliomas. On 8 1p/19q-deleted oligodendrogliomas, presenting angiogenesis and/or necrosis, 6 had a heterozygous and 2 a homozygous deletion of 9p. Three of them also had methylation of p16, which in one case was associated with a p16 mutation and in another to p14 methylation. These results illustrate the implication of 9p-deletion in angiogenesis and tumor necrosis of 1p/19q-deleted oligodendrogliomas and the putative role of p16 in these tumours. (vikkula@bchm.ucl.ac.be) (<http://www.icp.ucl.ac.be/vikkula>).

Homozygosity Mapping in familial glioma. *B.S. Malmer¹, H. Gronberg¹, S. Haraldsson², R. Henriksson¹, D. Holmberg².* 1) Dept Radiation Sci, Oncology, Umea Univ Hosp, Umea, Sweden; 2) Umea center for Molecular Medicine, Umea University Hosp, Umea, Sweden.

Background: The aetiology of primary brain tumours is in most cases unknown. Previous studies have identified a familial aggregation of glioma apart from the well characterised hereditary glioma-prone syndromes as Li-Fraumeni and Neurofibromatoses. About 5% of all glioma patients have a family history of gliomas in the family. In many of these families, siblings are described and we have previously performed a segregation analyses indicating an effect of an autosomal recessive gene. Material and Methods: Seven families from Northern Sweden were included in a homozygosity-mapping project. The families came from the same area in Sweden so the pedigrees were extended back to the 17th century to investigate if there were a common ancestor. Results: Three families were remotely related in several branches of the pedigrees indicating a possible founder mutation. Since autosomal recessive inheritance was suspected a homozygosity mapping project was initiated. The project is still ongoing and closer results will be presented at the meeting. Conclusions: Previous studies of hereditary cancer have focused on an autosomal dominant inheritance and mutation spectra in tumour suppressor genes. We have initiated a first study of familial cancer searching for autosomal recessive genes as a cause of hereditary cancer.

p14, p15 and p16 are frequently and differentially methylated in ependymal tumours. *E. Rousseau*^{1,2}, *M.M. Ruchoux*³, *F. Scaravilli*⁴, *F. Chapon*⁵, *C.A. Maurage*³, *C. Godfraind*², *M. Vikkula*¹. 1) Laboratory of Human Molecular Genetics, Christian de Duve Institute of Cellular Pathology and Université catholique de Louvain, Brussels, Belgium; 2) Laboratory of Neuropathology, Université catholique de Louvain, Brussels, Belgium; 3) Laboratory of Neuropathology, Hôpital Roger Salengro, Lille, France; 4) Department of Neuropathology, Institute of Neurology, London, UK; 5) Laboratory of Neuropathology, CHU Caen, France.

Several studies implicate numerical abnormalities of chromosome 9 in the pathogenesis of ependymal tumors, yet the three important tumor suppressor genes located in this chromosome, p16 (p16, CDKN2a, MTS1), p14 (p14ARF) and p15 (p15, CDKN2b, MTS2) are thought not be involved in the pathogenesis of these tumors. However, no promoter methylation analysis of these genes have been reported for ependymal tumors up to now, and because methylation is an important mechanism of gene silencing, we decided to study promoter methylation of p14, p15 and p16 in a series of 152 ependymal tumors by methylation-specific polymerase chain reaction. We observed that at least 76% of the studied ependymal tumors showed methylation for one of these three genes, and most tumors were methylated for only one gene. Low grade tumors present a high rate of methylation whatever the gene, implicating that methylation could be an early event in the development of ependymal tumors. There was a decrease in the percentage of methylated tumors when their grade increased for p15 and p14, and there were variations in the patterns of methylation for p14 and p15 regarding tumor location. The high frequency of methylation observed, and the variation in the patterns of methylation for tumors according to grade and location point to an important role of these three genes in the development of ependymal tumors, a fact which was not evident from the previous studies of these genes in ependymal tumors. (vikkula@bchm.ucl.ac.be) (<http://www.icp.ucl.ac.be/vikkula>).

Loss of heterozygosity in normal tissue adjacent to primary breast carcinomas. *D.L. Ellsworth¹, R.E. Ellsworth¹, B. Deyarmin¹, V. Mittal¹, S. Lubert¹, C.D. Shriver², R.I. Somiari¹.* 1) Clinical Breast Care Project, Windber Research Institute, Windber, PA; 2) Clinical Breast Care Project General Surgery Service, Walter Reed Army Medical Center, Washington, D.C.

Morphologically normal cells adjacent to breast carcinomas are known to harbor genetic abnormalities commonly observed in malignant tissues. It is unclear, however, whether genetic changes in adjacent normal cells reflect random mutational events or coordinated patterns of genetic change characteristic of the neighboring tumors. In this study, laser capture microdissection of paraffin embedded breast tissue samples was used to define patterns of loss of heterozygosity (LOH) in different classes of breast cancer, including ductal carcinomas in situ, lobular carcinomas in situ, papillary carcinomas, and infiltrating ductal carcinomas, as well as in surrounding morphologically normal stromal and epithelial cells. High-throughput fluorescent genotyping of 52 anonymous microsatellite markers was conducted to survey 26 chromosomal regions commonly deleted in breast cancer. In situ carcinomas showed rates of LOH greater than those in more advanced invasive tumors, and a higher prevalence of LOH was observed in normal cells adjacent to in situ carcinomas (63%) than in cells adjacent to invasive lesions (38%). LOH in adjacent normal cells was most prevalent on chromosomes 2, 3, 5, 6, and 13. Interestingly, patterns of LOH in normal stromal and epithelial cells differed from those in the neighboring tumors in ~50% of the cases examined. Patterns of chromosomal alterations in normal tissue adjacent to breast carcinomas may identify molecular events underlying the early stages of breast cancer development and help define tissues prone to cancer recurrence.

HPV18 Preferentially integrates at or near c-myc in HPV18-positive cervical tumors. *M.J. Ferber*^{1,2}, *W.F. Wu*³, *A. Brink*⁴, *E. Thorland*^{1,2}, *D.I. Smith*². 1) Biochemistry/Molec Biol, Mayo Foundation, Rochester, MN; 2) Division of Experimental Pathology, Mayo Foundation, Rochester, MN; 3) The Chinese University of Hong Kong, Hong Kong; 4) Leiden University Medical Center Department of Molecular Cell Biology Wassenaarseweg 72 2333 AL Leiden The Netherlands Leiden University Medical Center Department of Molecular Cell Biology Wassenaarseweg 72 2333 AL Leiden The Netherlands Leiden University Medical Center Department of Molecular Cell.

Virtually all cervical cancers are associated with human papillomavirus (HPV) infection. In addition, HPV integration is temporally associated with the development of invasive cervical cancer. We previously demonstrated at the molecular level that integration of one of the high risk HPV subtypes, HPV16, occurs preferentially within common fragile site (CFS) regions of instability (Thorland et al., *Cancer Research* 2000; 60:5916-5921). We then subsequently examined many more HPV16 integrations and found that 50% of the integrations occurred within a CFS region, and that there were several regions that contained multiple HPV16 integrations. We have now analyzed 20 HPV18-positive cervical tumors and again used restriction site oligonucleotide (RSO) PCR to rescue human sequences flanking the sites of HPV18 integration. The sites of integration were distinct from those observed with HPV16-positive cervical tumors. In addition, we found that 25% of the integrations occurred at 8q24 at or near the c-myc oncogene. Due to the previous association between HPV integrations and CFS regions, we examined the c-myc region in greater detail. C-myc is actually flanked by two CFS regions, FRA8C (8q24.1) and FRA8D (8q243). Using a FISH-based assay to characterize these two CFS regions and BAC clones that span c-myc, we have found that c-myc is localized in the region between these two CFSs. We are currently defining these two flanking CFS regions to determine their size and their structure. The presence of two CFS regions around c-myc may offer an explanation as to why c-myc is such a frequent target of amplification in cancer.

High-Throughput TRAP/PCR analysis of telomerase using capillary electrophoresis. *D.H. Atha¹, K. Miller², A.D. Sanow², J. Xu², J.L. Hess², O.C. Wu¹, W. Wang³, S. Srivastava³, W.E. Highsmith².* 1) Biotechnology Division, National Institute of Standards and Technology, Gaithersburg, MD; 2) Department of Pathology, University of Maryland School of Medicine, Baltimore, MD; 3) Division of Cancer Prevention, National Cancer Institute, Rockville, MD.

The enzyme telomerase is expressed in (85-90%) of all human cancers, but not in normal, nonstem cell somatic tissues. Clinical assays for telomerase, particularly in easily obtained body fluids, would have great utility as non-invasive, cost-effective methods for the early detection of cancer. The most commonly used method for the detection and quantification of telomerase enzyme activity is the polymerase chain reaction (PCR) based assay known as the telomeric repeat amplification protocol or TRAP assay. Most of the TRAP assay systems use a slab-gel based electrophoresis system to size and quantify the PCR-amplified extension products. We are developing high-throughput capillary electrophoresis (CE) methods for the analysis of TRAP/PCR products. The TRAP assay was conducted on lysates of the human lung cancer cell line A-549 in reactions containing 5 to 100 cells. Trap/PCR products were generated using a fluorescent (4,7,2'4'5'7',-hexachloro-6-carboxyfluorescein)-labeled TS primer and analyzed on the Applied Biosystems Model 310 CE system using POP4 polymer. After analysis with GeneScan and Genotyper software, the total peak area of the TRAP ladder extension products were computed using Microsoft Excel. Results were compared with unlabeled TRAP/PCR products analyzed on the Bio-Rad BioFocus 3000 CE system using 6% high molecular weight polyvinylpyrrolidone (HMW PVP) polymer and SYBR Green I dye. Both CE systems were able to resolve the TRAP ladder products with high reproducibility and sensitivity (5-15 cells). With the appropriate multi-capillary and robotic sample handling systems, these CE methods would streamline clinical validation of the telomerase TRAP assay with increased sensitivity, reproducibility and automation over slab-gel methods.

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Molecular characterization of a constitutional t(5;6)(q21;q21) in a patient with bilateral Wilms' tumor. C.D.

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Wilms' tumor, one of the most common pediatric tumors, is believed to arise from persistent metanephric blastema cells that fail to differentiate. Although the *WT1* gene (11p13) was identified more than a decade ago, it remains the only tumor suppressor gene known to be involved in Wilms tumor; furthermore, *WT1* is mutated in less than 10% of cases. Analysis of chromosomal rearrangements, particularly translocations, has been extremely useful in the identification of genes involved in cancer. Our lab has been investigating a balanced constitutional translocation in a patient with bilateral Wilms' tumor. The breakpoints of this t(5;6)(q21;q21) have been previously mapped between *D5S495* and *D5S433* on chromosome 5 and between *D6S301* and *D6S447* on chromosome 6, genetic distances of 4 cM and 3 cM, respectively (Hoban et al., J Med. Genet. 34:343-345, 1997). Using BAC clones selected from the interval between these flanking markers as FISH probes, we have narrowed the breakpoint region to approximately 100 kb by identifying BACs from both chromosomes 5 and 6 that span the translocation breakpoints. The derivative chromosomes have also been segregated into somatic cell hybrids to facilitate molecular analysis of the chromosome rearrangements. Three candidate transcripts, a known gene, an EST, and a predicted gene, map within the breakpoint regions. These genes are being assessed for changes in structure and/or expression resulting from the translocation. Analysis of our large Wilms tumor tissue collection and other tumor types will establish the involvement of one or more of these genes in human cancers. Funded by NCI/NIH CA63333 to MJH.

The Association of Cytoplasmic Overexpression of Cyclin D1 and Tumor Metastasis in Hepatocellular

Carcinoma. *L. Hu¹, J.M. Wen², W.S. Wang³, J.S.T. Sham¹, Y. Wang⁴, M.C. Wu⁴, D. Xie¹, D.J. Tang¹, X.Y. Guan¹.* 1) Clinical Oncology, Univ Hong Kong, Hong Kong, China; 2) Department of Pathology, Sun Yat-sen University, Guangzhou, China; 3) Department of Hepatobiliary Surgery, First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China; 4) Eastern Hepatobiliary Surgery Hospital, The Second Military Medical University, Shanghai, China.

Hepatocellular Carcinoma (HCC) is one of the worldwide most common malignant tumors with poor prognosis. In the present study, a marker chromosome containing a homogeneously staining region (HSR) in a recently established metastatic HCC cell line (H4-M) was characterized by comparative genomic hybridization and chromosome microdissection. The result showed that the HSR was composed of DNA sequence from 11q13 and amplification of cyclin D1 (CCND1) in H4-M was confirmed by fluorescence in situ hybridization (FISH) using a BAC clone containing CCND1 gene. Amplification and overexpression of CCND1 in H4-M has been demonstrated by Southern blot, Northern blot, and Western blot analyses. Immunohistochemical staining showed that the overexpression of CCND1 was located in cytoplasm in H4-M. Further study using a tissue microarray with 320 HCC samples showed that cytoplasmic overexpression of CCND1 was significantly higher in HCC with metastasis (19/56 cases, 34%) than that in HCC without metastasis (23/186 cases, 12%) ($P < 0.001$). This finding strongly suggested that the cytoplasmic overexpression of CCND1 may play an important role in the metastasis of HCC.

Genome-wide search for homozygous deletions in oral cancer. *H. Kayahara*¹, *H. Yamagata*^{2, 3}, *T. Miyoshi*¹, *M. Abe-Ochi*³, *J. Nakura*³, *I. Kondo*², *T. Miki*³, *H. Hamakawa*¹. 1) Dept Oral and Maxillofacial Surgery, Ehime Univ Sch Medicine, Onsen-gun, Ehime, Japan; 2) Dept Hygiene, Ehime Univ Sch Medicine, Ehime, Japan; 3) Dept Geriatric Medicine, Ehime Univ Sch Medicine, Ehime, Japan.

To date, in head and neck cancer including oral squamous cell carcinoma (OSCC), loss of heterozygosity (LOH) has been identified on chromosomes 2q, 3p, 4q, 7q, 8p, 9p, 10q, 11q, 13q, 14q, 17p, 18q and 22q. These findings suggest that some of these regions may contain a tumor suppressor gene. However, the most studies failed to isolate the candidate genes, except for DPC4 in colon cancer and PTEN in breast cancer that came from the mapping of homozygous deletions. To identify the putative tumor suppressor gene locus, we analyzed 6 cell lines from OSCC with 811 microsatellite markers (ABI PRISM Linkage Mapping Sets LMS-HD5) covering the entire chromosome except Y for allelic changes. Multiplex PCR products were electrophoresed on an ABI PRISM 3100 Genetic Analyzer and the fluorescent signals from the different sized alleles were recorded and analyzed by Genotyper and GeneScan. Homozygous deletions were found in 38/811 markers (4.7%) on average. Among them, 26 markers were screened for further studies in 12 OSCC cell lines. We identified three homozygously deleted regions in 12 cell lines. The candidate gene loci were as follows: D6S292 (6q23.2), D10S192 (10q24.32), and D18S68 (18q22.1). At present, no gene deletions have been identified in the centromeric and telomeric boundary of these loci.

Study of chromosomal abnormalities in esophageal squamous cell carcinoma by comparative genomic hybridization. *A.L.S. Tai, D.L.W. Kwong, J.S.T. Sham, X.Y. Guan.* Clinical Oncology, The University of Hong Kong, Hong Kong, China.

Esophageal carcinoma ranks among the nine most common cancer worldwide. In China, its incident rate is particularly high compare to the western countries. Squamous cell carcinoma is the dominant histological type found in the Asian populations. In this study, comparative genomic hybridization was used to screen for the genomic alterations among 60 primary esophageal squamous cell carcinoma cases globally. Chromosomal aberrations were detected in 52 cases. The frequent chromosomal gains were detected in 3q (67.3%), 8q (57.7%), 5p (51.9%), 7q (28.8%), 15q (28.8%), 20q (28.8%), 20p (21.1%), 1q (26.9%), 7p (26.9%), 2p (23.1%) and 12p (23.1%), where the chromosomal losses involved 3p (46.2%), 4q (26.9%), 4p (23.1%), 3q (19.2%), 9p (17.3%) and 13 (15.4%). High copy number amplifications were found in 3q and 8q among 10 and 8 cases, respectively, with minimum overlapping regions of 3q26.1-26.2 and 8q24.1-24.2. Interphase-FISH, using BAC clones was used to study the expression of eIF-5A2 and myc. Amplification of both eIF-5A2 and myc was found. In summary, genomic changes are common in esophageal squamous cell carcinoma.

Molecular FISH markers for metastasis in ductal breast carcinoma. *H. Zhao, J.G. Jones, H.P. Klinger, B. Vikram, P.M. Achary.* Albert Einstein College of medicine, Bronx, NY.

The objective of this project is to construct a panel of molecular genetic markers for detecting those 13% of breast cancer patients with negative lymph nodes, so that they could be treated more aggressively. Representational Difference Analysis was used to compare the DNA of cells from archival normal tissue or primary ductal tumor with that of the metastatic lymph node of the same patient in order to isolate those sequences that were lost in the course of tumor metastasis. The tumor cells were recovered by laser capture microdissection. We isolated 11 sequences that are candidates for metastasis associated genes (MAGs) because they were lost in metastatic cells. To-date three of these 11 sequences were used to screen normal, primary and metastatic cell DNA samples. MAGS-XI was found to be lost in the metastatic cells of 3 out of the 5 tumors. MAGS-IX was found to be lost in metastases from 2 out of 5 primary tumors, and MAGS-IV was lost in 1 out of 3 tumors. RH mapping and homology search results indicated that MAGS-IX was located on the long arm of chromosome 10 where the PTEN, a known metastasis suppressor gene is also located. To determine if MAGS-IX is perhaps a part of the PTEN gene we PCR screened the above mentioned five tumor cell DNA samples and a breast carcinoma cell line, HCC-1937, which has homozygous loss of the PTEN gene. The results indicated that MAGS-IX is a novel gene sequence. Presently we are isolating partial and/or full-length sequences of these MAGS to use as fluorescence in situ hybridization (FISH) probes to screen a larger number of tumor samples. A 2Kb sized MAGS-IX has been generated and localized to the q21 region of human chromosome number 10 by FISH. Screening of MAGS-IX as fish probe in the primary tumor tissue sections of a breast carcinoma which metastasized to lymph nodes showed nuclei with signals indicating normal, heterozygous and homozygous losses of MAGS-IX in the ratio of 11:13:1 respectively. We conclude that the MAGS-IX could possibly be used as a FISH probe to identify primaries that are prone to develop metastasis.

***MLH1* exon 3 deletion in cDNA associated with 213_215delAGA: Probable exon splicing enhancer mutation as a cause of HNPCC.** G. Chong^{1,2,5}, G. Ouellette⁶, B. Lemieux⁶, V. Marcus³, I. Thiffault¹, E. MacNamara^{2,3,5}, W.

Foulkes^{1,4}. 1) Department of Human Genetics; 2) Department of Medicine; 3) Department of Pathology; 4) Program in Cancer Genetics, McGill University, Montreal, QC, Canada; 5) Diagnostic Medicine Department, SMBD-Jewish General Hospital, Montreal, QC, Canada; 6) Medical Genetics Service, University of Sherbrooke Medical Centre, Sherbrooke, QC, Canada.

We describe a new mutation in *MLH1* in an Amsterdam criteria I-fulfilling HNPCC kindred. The proband is a 53 yr old woman who was diagnosed with colon cancer at age 49. Her brother was diagnosed with rectal cancer at 39 and died at age 48. Her son died of colon cancer at 28. IHC showed loss of MLH1 protein in the two available colon cancers. PTT revealed an abnormal truncated protein. RT-PCR analysis of her cDNA revealed a shortened product, which on sequencing was found to be caused by the entire in-frame deletion of exon 3. Sequencing of the genomic DNA did not detect any splice site variant which might have explained the deletion of exon 3 in the cDNA. However, a 3 bp deletion at nt 213, predicted to result in DE71, was detected. The same deletion was found in the tumor from her son, with LOH. These findings suggest that the exon 3 deletion has resulted from the 213_215delAGA genomic alteration. The sequence around the mutation is purine-rich (AAAGAAGAT), and as these have been associated with exon splicing enhancers (ESEs), we postulate that 213_215delAGA has removed an ESE for exon 3. An in-frame deletion in exon 7 of SMN has been previously associated with aberrant splicing of this exon¹ and is thought to be disease-causing. Similar ESEs (-AAGAAGA-) have also been identified in the genes for human fibronectin (exon ED1)² and calcitonin (exon 3)³. As the significance of our mutation would not have been appreciated without examination of both DNA and RNA, this finding illustrates one benefit of multi-modal molecular screening for mutations in mismatch repair genes.

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INK4/ARF germline mutations and additional neoplasia in pancreatic cancer patients and their families. L. Bonelli¹, P. Ghiorzo², L. Pastorino², R. Cusano¹, A.M. Nicora¹, S. Zupo¹, P. Queirolo¹, M.R. Sertoli², V. Pugliese², G. Bianchi-Scarrà². 1) Clinical Epidemiology Unit, Natl Inst for Cancer Research, Genova, Italy; 2) Dept Oncology Biol. Genetics, Univ. of Genova, Genova, Italy.

Roughly 40% of germinal mutations in melanoma families (MF) affect p16INK4a (p16) and p14ARF (p14). We previously found an excess of pancreatic cancer (PC) in MF carrying the G101W founder mutation. We now look at: the role of mutations in p14 among non-p16 MF; the role of p16 and p14 in sporadic PC (SPC) patients; the incidence of other tumors in SPC patients' families. We studied 49 MF enrolled between '95 and '01 at the Medical Genetics Service, 66 PC patients diagnosed at the NCI in Genoa between '98 and '01 and 54 controls. The MF consisted of 967 relatives, with 118 melanoma and 10 PC patients. The 18 G101W MF had an increased risk of developing PC (RR=9.4; 95%CI 2.7-33.4), unlike the 31 wt (non-p16, non-p14) families (RR=2.2; 95% CI 0.8-5.7). Median age at onset of PC in mutated families was 57.5 yrs (mean 61, range 49-78) vs. 77.5 in wt kindreds (mean 77, range 65-88), the same as found in the local Cancer Registry (mean 77, range 75-79). Sixty-one SPC patients and 54 controls were eligible; an in-person interview was conducted with 46/61 patients and 52/54 controls, for the others a kin was interviewed. Incidence of tumors in 836 blood relatives of patients vs. 711 of controls showed no significant difference. Tumor clusterings were identified in 17 branches from as many families of PC patients vs. 7 of controls. Some showed part of the tumor spectrum associated with family cancer syndromes. In particular, 2 showed a melanoma-PC association in concert with G101W p16 mutation, although no member displayed an atypical mole phenotype. No mutation was found in controls. The increased risk of PC in G101W carriers but not in wt families and cases does not rule out that coexisting alterations in p16 and p14 may be involved in the development of PC in those patients. The presence of G101W in 2/46 SPC patients confirms the prevalence of our founder mutation and suggests that relatives of PC patients may benefit from melanoma surveillance programs. Supported by Ministry of Health NICS0701/RF99.63 to GBS.

Hypermethylation of the CpG islands in the promoter region flanking GSTP1 gene is a candidate plasma DNA biomarker for screening or monitoring prostate carcinoma prognosis. *D. Chu*^{1, 3}, *C. Chuang*², *J. Fu*³, *S. Huang*², *R. Chia*³, *C. Sun*³. 1) School of Medical Technology, Chang Gung University, Tao-Yuan, Taiwan; 2) Division of Urology, Department of Surgery, Chang Gung Memorial Hospital, Lin-Kou, Taiwan; 3) Laboratory of Molecular Diagnosis, Department of Clinical Pathology, Chang Gung Memorial hospital, Lin-kou, Taiwan.

Purpose: to investigate whether our newly developed methylation-sensitive real-time PCR protocol could be used to detect cancer genome in prostate cancer patients' circulations. **Study design:** Serum or plasma DNA samples were collected from twenty-nine prostate cancer patients and thirty-six benign prostate hyperplasia (BPH) cases. After extensive methylation-sensitive restriction enzyme, *HpaII*, digestion, the DNA samples were subjected to the real-time PCR amplification. The amplification curves were adopted to know if hypermethylation occurred in the promoter region flanking the GSTP1 gene among these prostate cancer patients. **Results:** If we use the previously used cut-off cycle of threshold (Ct) value of 35, 11 of 29 prostate cancer patients could be correctly identified. However, none of the 36 BPH cases showed positive (Ct<35) results. To our attention, there were 15 prostate cancer cases did not have detectable cancerous genome in the plasma or serum. **Conclusions::** We have successfully analyzed prostate cancer genome in the peripheral blood in prostate cancer patients with the highly sensitive protocol. Due to the fact that the analytical results depended on whether enough cancer genomes presented in the plasma or serum sample, this protocol performed on plasma or serum is more suitable for mass-screening of prostate neoplasm or for follow-up after treatments.

Detection of *BRCA1* rearrangements in breast-ovarian cancer families using Quantitative Multiplex PCR of Short Fluorescent Fragments (QMPSF). *F. Casilli*^{1, 2}, *I. Tournier*¹, *Z.-C. Di Rocco*², *S. Gad*³, *D. Stoppa-Lyonnet*³, *T. Frebourg*¹, *M. Tosi*¹. 1) INSERM EMI 9906, Faculty of Medicine, Rouen, France; 2) Department of Experimental Medicine, University L'Aquila, 67100 Coppito, Italy; 3) Department of Genetics, Institut Curie, 26 rue d'Ulm, 75005 Paris Cedex 5, France.

Twenty different *BRCA1* rearrangements have already been described and the contribution of this type of defects is considered to be significant in breast-ovarian cancer families with high probability of *BRCA1*- or *BRCA2*- linked predisposition. The difficulty of detecting such heterozygous exon deletions or duplications has stimulated the development of several new screening methods. Quantitative Multiplex PCR of Short fluorescent Fragments (QMPSF) is based on simultaneous amplification of multiple short target sequences under conditions that allow rapid and reliable quantitative comparison of the fluorescence of each amplicon in test samples and in controls. We have developed, as a routine screening test, QMPSF for *BRCA1*. All *BRCA1* coding exons are analysed using four multiplexes. Analysis, using QMPSF of families without point mutations in the exons or splice-sites of *BRCA1* and *BRCA2* and selected because of high probability of a *BRCA1*- or *BRCA2*- linked genetic predisposition allowed us to identify several rearrangements including two novel deletions, which respectively involved exons 15-16 and exons 1-22. The 1-22 deletion is the largest exonic deletion found so far within the *BRCA1* gene. This rapid, sensitive and cost-effective method is also being adapted to the screening of *BRCA2* and may be included into the routine molecular analysis of breast-ovarian cancer predispositions.

Detection of the first complete heterozygous deletion of the *TP53* gene in a family with Li-Fraumeni syndrome. G.

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The Li-Fraumeni syndrome (LFS) represents one of the most devastating genetic predispositions to cancers and is characterized by a wide spectrum of early-onset malignancies including bone and soft-tissue sarcomas, brain tumours, adrenocortical tumours and pre-menopausal breast cancers. The absence of detectable germline *TP53* mutation in some LFS families has suggested the involvement of other genes, but this remains controversial. The density of *Alu* repeats within the *TP53* gene led us to screen for genomic rearrangements of *TP53* in families without detectable *TP53* mutation. To this aim we adapted the QMPSF (Quantitative Multiplex PCR of Short fluorescent Fragments) to the analysis of the 11 exons of *TP53*. We analyzed 100 families, either fulfilling or partially meeting the criteria for LFS and in which classical methods had revealed no *TP53* alteration. We identified, in one extended family fulfilling the criteria for LFS, a complete heterozygous deletion of *TP53*. Additional QMPSF analyses of genes surrounding the *TP53* locus showed that the deletion, estimated to 30-40 kb, involved only the *TP53* locus. This study shows that the occurrence of germline rearrangement of *TP53* is a rare event but must be considered in families fulfilling the original criteria for LFS and without detectable *TP53* mutation.

Conventional and Tissue MicroArray Immunohistochemical Expression Analysis of Mismatch Repair in Hereditary Colorectal Tumours. A. Bröcker-Vriends¹, Y. Hendriks¹, P. Franken¹, J.W. Dierssen², W. de Leeuw², J. Wijnen¹, C. Tops¹, M. Breuning¹, H. Vasen³, R. Fodde¹, H. Morreau². 1) Depts. of Human and Clinical Genetics; 2) Pathology, Leiden Univ. Medical Center; 3) Foundation of Hereditary Tumours, Netherlands.

Background and aims HNPCC is caused by germline mutations in the DNA mismatch repair (MMR) genes. IHC of MMR proteins and MSI in HNPCC related tumours can be used as pre-screening methods. Aims of the study: 1) Determination of the sensitivity and specificity of IHC for MLH1, MSH2 and MSH6 mutations, 2) Evaluation of the validity of IHC-TMA (Tissue MicroArray) compared with IHC-WS (Whole Slide).

Methods IHC-WS was performed in crc of 45 carriers of a MLH1, MSH2 or MSH6 mutation. The TMA cohort consisted of these 45 tumours and 84 crc from patients with S-HNPCC.

Results IHC-WS showed abrogation of at least one of the three MMR proteins tested in 86% (18/21), 100% (12/12), and 75% (3/4) of tumours from carriers of a MLH1, MSH2 and MSH6 mutation resp. Mismatch repair deficiency would thus have been detected in 89% (33/37) of the cases. MSI analysis was slightly more sensitive than IHC (25/27, 93%). For MLH1, MSH2 and MSH6, IHC-WS would have correctly predicted a mutation in 48%, 92% and 75% of the cases resp. Comparison of the IHC results based on whole slides versus TMA, showed a high level of concordance for MLH1 (85%) and MSH2 (95%), a slightly lower level for MSH6 (75%).

Conclusions The results of our study show that both IHC and MSI are sensitive pre-screening methods in identifying patients for MMR mutation analysis. IHC has the additional advantage of identifying the respective MMR gene involved. We propose a scheme for the diagnostic approach of families with (suspected) HNPCC. This study also shows that high-throughput IHC analysis performed by Tissue Micro Arrays can be reliably employed to simultaneously screen a large number of tumours from (suspected) HNPCC patients for MMR protein alterations.

Demographics and phenotype of familial Barrett's esophagus and esophageal adenocarcinoma series. C.M.

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Barrett's esophagus (BE) is a metaplastic columnar lined esophageal epithelium thought to be the result of a continual reparative process in response to chronic gastric reflux. BE is often a precursor of esophageal adenocarcinoma (EAC) and is most often diagnosed in males over age 50. Familial factors in development of BE/EAC have long been recognized. It is likely that inheritance of a BE/EAC predisposition follows the autosomal dominant model of most inherited cancer syndromes, but no causative gene has yet been identified. Our consortium has compiled the largest series of families with BE/EAC yet reported. Families were recruited from consortium sites and self-referral from advertisement. Family histories were taken through interview with the proband and considered positive if two or more individuals were diagnosed with BE/EAC. Available pathology and/or endoscopy reports and tissue samples were reviewed. Pedigrees were drawn and data entered for a total of 65 families comprising 156 affected and 390 unaffected individuals. Mean ages of diagnosis of BE and EAC among females were 48.7 and 60.2 years, and among males were 51.7 and 55.6 years, respectively. In contrast, typical ages at diagnosis for sporadic BE and EAC are approximately 63 and 64 years, respectively, for both sexes. Ten percent of affected and 15.2% of unaffected individuals were diagnosed with a cancer other than EAC. Power simulations were performed to estimate the expected lod score using typical pedigree structures in this data and four different autosomal dominant models that are consistent with the previously reported BE/EAC population prevalence and sibling risk ratio. Assuming at least two loci, a PIC of 0.7 at the marker locus, and theta = 0.05 in a 400 marker genome scan, 120 families are needed to achieve a lod score of 3.0 for a linked locus. Identification of a causative gene would enable predictive testing for BE/EAC, and is likely to give clues to the molecular pathogenesis of BE and EAC as well as other cancers.

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B-RAF as a melanoma susceptibility candidate gene. *B. Bressac-de Paillerets, K. Laud, M-F. Avril, A. Chompret, G. Lenoir and French Hereditary Melanoma Study Group.* Inst Gustave Roussy, Villejuif, France.

The mitogen-activated protein (MAP) kinase cascade are membrane-to-nucleus signaling modules involved in multiple physiological processes. The Ras-RAF-MEK-ERK-MAPKinase pathway mediates cellular responses to growth signals. In a melanocyte-specific signaling pathway, B-RAF mediates the c-AMP-activation of ERK. As B-RAF somatic missense mutations have been shown to occur in 66 percent of malignant melanoma with 80 percent of mutations being a single substitution (exon 15, V599E), we hypothesize that B-RAF could be a melanoma susceptibility gene. Therefore, we searched for B-RAF germline mutations by direct sequencing of the entire B-RAF coding region (18 exons) for 12 highly selected melanoma-prone families. We also screened exons 11 and 15 in a large series of 45 melanoma-prone families or individuals. Results of our analysis will be presented.

Prevalence of BRCA1 and BRCA2 mutations in ovarian cancer patients from the French-Canadian population.

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Studies on high-risk breast and ovarian cancer families have established the spectrum of BRCA1 and BRCA2 mutations present in the French-Canadian population. Two founder mutations have been reported in this population, one in each of the two BRCA genes. As ovarian cancer is the second leading cause of death from cancer in women, a better understanding of molecular alterations should contribute to improve prevention strategies and treatment, therefore reducing mortality due to ovarian cancer. To evaluate the incidence of BRCA1 and BRCA2 mutations in ovarian cancer, eighteen different mutations (10 in BRCA1 and 8 in BRCA2), reported at least once in French-Canadian ancestry, have been sequenced in a series of nearly 70 ovarian tumors not selected on the basis of a family history, from the Ovarian Cancer Clinic at Hotel-Dieu de Quebec, Canada. We identified two tumors bearing the BRCA1 founder mutation R1443X and one with the BRCA2 founder mutation 8765delAG. Both R1443X mutations were found at a homozygous state in the tumor, consistent with loss of heterozygosity. Another previously reported mutation was found, the 2953del3+C in BRCA1. In addition to these three mutations, two other changes have been found in two different tumors, a deletion of 13 and 7 nucleotides at position 1078 and 3721, respectively. To our knowledge, these two changes have not been reported previously and we are currently characterizing these two deletions in normal tissues coming from the same patients. In conclusion, alterations in BRCA1 or BRCA2 have been identified in approximately 9% of ovarian tumors, while the two most frequent BRCA1 and BRCA2 French-Canadian founder mutations have been found in approximately 5% of ovarian tumors. These findings could thus have clinical implications for adequate follow-up of ovarian cancer patients from the French-Canadian population.

Prevalence of BRCA1 and BRCA2 mutations on full sequence analysis of women with invasive breast cancer diagnosed in their fifties. C.A. Frye, H.L. Shappell, A.M. Deffenbaugh, L.A. Burbidge, T.S. Frank. Myriad Genetic Laboratories, Salt Lake City, UT.

An individual's age at diagnosis of invasive breast cancer is regarded as one of the primary factors in the risk assessment of hereditary breast and ovarian cancer syndromes. The earlier the diagnosis of invasive breast cancer the more likely the cancer is due to a *BRCA1/2* mutation. Questions remain regarding the appropriateness of *BRCA1/2* testing in individuals presenting with invasive breast cancer in their fifties. The aim of this study was to address the prevalence of mutations identified by full sequencing of the *BRCA1* and *BRCA2* genes in women diagnosed with invasive breast cancer after the age of 50, and to correlate the presence of such mutations with features of the family history. Overall deleterious mutations were identified in 121 of 1,327 (9.1%) women diagnosed with breast cancer between ages 50 and 59, including 54 in *BRCA1* and 68 in *BRCA2*. One individual had two deleterious mutations, one in *BRCA1* and one in *BRCA2*. Mutations were identified in 52 of 497 (10.5%) probands who indicated at least one first or second degree relative with invasive breast cancer diagnosed before the age 50, and in 15 of 155 (9.7 %) who indicated at least one first or second-degree relative with ovarian cancer at any age. In contrast, mutations were identified in 10 of 248 (4.0 %) probands who indicated at least one first or second degree relative with invasive breast cancer diagnosed after the age 50. Women diagnosed with invasive breast cancer in their fifties should be offered the option of genetic testing for *BRCA1/2* mutations, especially if their family history includes a diagnosis of breast cancer before age 50, or ovarian cancer at any age, in a first or second degree relative.

Identification of inactivating large scale deletions or other gene rearrangements in the BRCA1 gene by VNTR analysis coupled with Long-Range PCR. *S. Fard, B. Fraser, P. Ainsworth.* Mol Dx Lab, LHSC and Dept. Biochem, Univ. Western Ont, London, Ontario, Canada.

Larger genomic rearrangements have been reported to constitute a substantial proportion of BRCA1 gene mutations^{1,2}. These types of mutations may be identified by loss of heterozygosity (LOH) analysis using variable number tandem repeats (VNTRs). To determine the frequency of BRCA1 deletion events in at risk samples, a series of VNTRs spaced across the BRCA1 gene have been characterised. 5 of 20 di-, tri-, and tetra-nucleotide repeats within the BRCA1 gene were shown to be polymorphic. The relatively low frequency of informative VNTRs identified precluded the direct use of these markers to effectively define hemizyosity resulting from a gene deletion. We have therefore used the VNTRs (as markers of heterozygosity) in conjunction with Long-Range PCR (LR-PCR) to identify smaller deletion and/or duplication events within the BRCA1 gene. The BRCA1 gene (~120 kbp) was amplified by LR-PCR in overlapping fragments ranging from 5 to 20 kbp, each encompassing at least one of the polymorphic VNTRs noted above. The PCR amplicons were sized by 0.8% agarose gel electrophoresis. For amplicons of a normal size, confirmation of heterozygosity of the VNTR(s) within the amplified LR-PCR product demonstrated amplification of both alleles and ruled out a genomic deletion. A series of DNA samples, from S.W. Ontario individuals at a high risk for familial breast/ovarian cancer, had been screened by the protein truncation test (PTT). From this series 50 samples which were non-positive by PTT, as well as a subset of samples that were screen positive by PTT, but in which the causative mutation was not identified, have been subjected to the VNTR/LR-PCR screen described above. A preliminary analysis of results, consistent with more recent literature³, appears to confirm that deletion events at the BRCA 1 gene locus are not a common cause of inactivation of the function of this gene in the South Western Ontario population. References: 1. Puget N. et al. (1999) *Cancer Research* 59:455-61 2. Swensen J. et al. (1997) *Hum Mol Genet* 6:1513-7 3. Lahti-Domenici J. et al. (2001) *Cancer Genet Cytogenet* 129:120-3.

Development of a Targeted *BRCA1/BRCA2* Mutation Screening Panel for African-American Women. *L. Gayol*¹, *M.E. Ahearn*¹, *L. Fernandez*¹, *E. Estrella*¹, *R. Mirhashemi*², *J.F. Arena*², *L. Baumbach*¹. 1) Pediatrics/Genetics, Univ.of Miami School of Medicine, Miami, FL; 2) FOBCC, Univ.of Miami School of Medicine, Miami, FL.

Recent evidence from our laboratory, as well as others, suggest that certain *BRCA1/BRCA2* mutations and other genetic variants appear to be either at increased frequency, or specific, for African-American (AA) women at-risk for breast cancer. We recently completed *BRCA1* and *BRCA2* mutation scanning in twenty-five at-risk AA families. *BRCA1* and *BRCA2* germ-line alterations were detected first using a series of exon-specific PCR primers for SSCP analysis, followed by DNA sequencing of SSCP variants. In this cohort, only one protein-truncating mutation was detected in either *BRCA1* or *BRCA2*, however, splice mutations, missense mutations, and a number of polymorphic variants were detected in both *BRCA1* and *BRCA2*, with a higher frequency occurring in *BRCA2*. Many of these variants were both novel, and specific to AA patients, as well as present in the absence of another disease-causing mutation. These results agree with previous observations that deleterious mutations in *BRCA1* or *BRCA2* are uncommon in at-risk AA patients, and suggest that additional benign variants should be further evaluated for their potential role in the disease process in AA patients. These observations have lead to the development of an screening panel for such *BRCA1* and *BRCA2* mutations/variants for AA women. This panel consists of thirteen mutations and thirteen unclassified variants in *BRCA1* and five mutations and six variants in *BRCA2*, either detected in our laboratory or found in a thorough review of all published English literature. The screening strategy is based on a combination of PCR and multiplex SSCP, as well as *GENESCAN* technology using an ABI 3100. Site-directed mutagenesis has been used to synthesize mutation-positive controls for each variant to be analyzed. We will present our latest progress in panel development and mutation screening. Implementation of this panel will contribute significant new information regarding the frequencies and conferred risks of mutations and variants in the African-American population.

Sequence variation in the MC1R gene and melanoma risk. *J.A. Douglas¹, J. Davis¹, T.R. Rebbeck², M. Berwick³, S.B. Gruber¹.* 1) Depts Hum Genet, Epid, & Int Med, Univ Michigan, Ann Arbor, MI; 2) Dept Biostat & Epid, Univ Penn, Philadelphia, PA; 3) Dept Epid & Biostat, Memorial Sloan-Kettering Cancer Center, New York, NY.

The purpose of this investigation was to evaluate the relationship between genetic variation in the MC1R gene and melanoma risk in an initial sample of 100 Caucasian Americans (75 cases and 25 spouse controls) by sequencing the entire 953 bp coding region. Sequencing showed variation in 15 sites: 14 single nucleotide substitutions (12 non-synonymous and 2 synonymous) and 1 insertion/deletion. MC1R variants were found in 80% of cases and only 52% of controls. Two variants, R151C and R160W, were significantly associated with melanoma risk ($p=0.013$ and $p=0.028$, respectively) and were observed in cases only at frequencies of 11% and 9%, respectively. The strength of linkage disequilibrium (LD) between R151C and R160W (as measured by D') was only 12% of its theoretical maximum and was expectedly non-significant via a likelihood ratio test ($p=0.516$). These results are consistent with recent epidemiological and functional findings demonstrating a reduction in melanin synthesis for both variants, although adjusted case-control analyses suggest that the effects of these variants are not entirely mediated by pigmentation, i.e., by differences in eye, hair, or skin color. There were no significant differences in allele or genotype frequencies for the remaining 13 sites, although only 3 had minor allele frequencies $>5\%$. Given exceptionally short inter-marker distances, few common alleles, and a small sample size, a majority of our sites were in complete pair-wise LD ($|D'|=1$), with relatively few site pairs in significant LD. These results underscore the importance of sample size and marker/mutation frequency on the power to detect LD and illustrate that less common variants (minor allele frequencies $<20\%$) may be important functional predictors of risk. With our relatively small sample size analyzed thus far, it is not possible to exclude a melanoma role for many of the less common MC1R variants, but our data are consistent with the hypothesis that R151C and R160W are predictors of melanoma risk.

From Research to Service: Analysis of Mutation Yield Comparing the Population-Based OFBCR and the Ontario Cancer Genetics Program's BRCA1/2 Genetic Testing Eligibility Criteria. *G.D. Glendon¹, S. Taylor², W. Meschino³, J. Allanson⁴, I. Andrulis⁵ and Ontario Cancer Genetics Program Implementation Committee & Ontario Cancer Genetics Network.* 1) OCGN, Cancer Care Ontario, Toronto, ON, Canada; 2) Kingston Gen. Hosp., Kingston, ON, CA; 3) Dept. Genetics, North York Gen. Hosp. Toronto, ON, CA; 4) Dept. Genetics, Children's Hosp. of Eastern Ontario. Ottawa, ON, CA; 5) Samuel Lunenfeld Inst., Mount Sinai Hosp., Toronto, ON, CA.

From 1996 to April 2000 the Ontario Cancer Genetics Network (OCGN) provided genetic testing for the BRCA1/2 genes on a research basis. At that time, the Ontario Ministry of Health and Long Term Care (MOHLTC) began providing support for BRCA1/2 genetic testing as a clinical service. The Clinical sub-group of the Cancer Genetics Program Implementation Committee developed the service testing eligibility criteria aiming to identify families with a 10% or greater probability of carrying a BRCA1/2 mutation. The Ontario Familial Breast Cancer Registry (OFBCR) is an incident population-based familial breast cancer registry created to foster familial breast cancer research. It is administrated by the OCGN and is one of six sites that comprise the NIH funded Breast Cancer Family Registries. BRCA1/2 mutation analysis utilizing protein truncation analysis with 5' DNA sequencing (PTT) was performed on a wide variety of OFBCR participants meeting specific personal and family history criteria. Thirty-one of the 315 (9.8%) OFBCR eligible participants tested in the 1996 ascertainment period had BRCA1/2 mutations. An analysis applying the service eligibility criteria to this cohort yielded 250 of the 315 as eligible for service testing, of which 28 (11.2%) would have had BRCA1/2 mutations. Of the 6 Ashkenazi Jewish individuals that the service criteria would have excluded due to late age at onset, 2 carried the 6174delT BRCA2 mutation. Results from the service program's actual BRCA1/2 testing (PTT) from Sept. 1, 2000 to Sept. 1, 2001 revealed that 120 of 988 (12.1%) families had BRCA1/2 mutations thereby meeting their stated goal. A total of 19 mutations were identified more than once and 55.5% of the mutations identified were in BRCA1.

Approximately 25% of non-syndromic, non-familial pheochromocytoma patients carry germline mutations.

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When a patient has an operational >10% a priori probability of a germline mutation which gives cancer susceptibility, clinical gene testing is offered. It has been assumed that >90% of all pheochromocytoma (PC) cases are sporadic. Genetic testing is not offered to such patients unless syndromic features or family history are present. In order to test the hypothesis that germline mutation in 1 of 4 PC susceptibility genes in apparently sporadic pheochromocytoma would occur >10% of the time, we analyzed 280 non-syndromic patients without a family history from the population-based German-Polish Registries presenting with symptomatic PC for germline mutations in RET (multiple endocrine neoplasia type 2), VHL (von Hippel-Lindau syndrome), SDHD (familial glomus tumors/paraganglioma) and SDHB (familial glomus tumors/paraganglioma). Comprehensive clinical studies performed include personal and family histories, physical exam, biochemical studies and imaging (eg, MRI) studies of the neck, thorax and abdomen. Of the 280 unrelated apparently sporadic cases, 67 (24%) were found to have germline mutation. Of the 67, 13 had RET, 31 VHL, 11 SDHD and 12 SDHB mutations. While the expected younger age and multifocal presentations were associated with germline mutations, 46 cases with single tumor presentations were mutation carriers and 11 presented after age 40. The youngest presentations (mean 17.7 y) were the most common with VHL (14y) and SDHB mutations (26y). We found that extra-adrenal tumors, eg paragangliomas and glomus tumors, were associated with germline mutation, in particular those in SDHD. Overall, 43 (64%) of the patients were identified solely by molecular testing for RET, VHL, SDHD and SDHB. In summary, because one fourth of apparently sporadic PC were found to carry germline mutations in 1 of 4 genes, routine genetic testing should be offered to all presentations of PC in order not to miss occult hereditary cases. The features at presentation might help suggest which gene to initiate testing.

Searching the germline *LKB1/STK11* mutation in the only known family affected with Peutz-Jegher Syndrome from the São Miguel island (Azores, Portugal). L. Mota-Vieira¹, F. Melo-Bento¹, C.C. Branco¹, J.M. Cabral¹, N. Nunes¹, Z. Rego¹, H. Brum¹, J. Gonçalves¹, H. Mehenni², A. Andrade¹. 1) Hospital of Divino Espirito Santo, Azores Islands, Portugal; 2) Laboratory of Human Molecular Genetics, Geneva University Medical School, Switzerland.

Peutz-Jeghers syndrome (PJS) is a rare autosomal dominant disorder with variable expression and incomplete penetrance. It is characterized by hamartomatous polyposis of the gastrointestinal tract and melanin pigmentation of both skin and mucous membranes. PJS patients also show a predisposition to a wide spectrum of cancers. To date, 78 germline mutations in the *LKB1/STK11* tumour suppressor gene (19p13.3) have been reported as the cause of PJS. Most of them occurred in only one or a few families, of which several have arisen *de novo*.

Here, we report a large PJS family from the Azorean island of São Miguel (Portugal). This family is the only PJS family registered in our Hospital (Azores central hospital). The family history revealed 13 patients (6 deceased), spanning 5 consecutive generations. Among the remaining 7 patients, 4 are adults with aged 30-35y (3rd and 4th generations) and 3 are children aged 4-6y (5th generation). They were all evaluated according to clinical protocols adjusted to patient's age (imagingologic, endoscopic and histologic exams- of the polyps or the necessary resections). Surprisingly, after a routine thorax X-ray exam, a neoplasia - a neuroendocrine tumor of T cells of the mediastinum - was found in one adult patient. This is the first case reporting the presence of this kind of tumor in PJS patient. In order to identify the causative germline mutation segregating in this family, DNA were isolated from blood samples of the selected 7 patients and 6 unaffected relatives. Evidence of linkage was identified with markers located on 19p13.3. We are carrying out the sequencing of the 9 exons and adjacent intronic sequences of *LKB1/STK11* gene. These patients are undergoing a clinical surveillance program. The 3 affected children are also undergoing clinical follow up adjusted to age. (lmv.hospdelgada@mail.telepac.pt).

Contribution of *MSH2*, *MLH1*, *MSH6* and *TGFBR2* Germline Mutations to Colorectal Cancer Susceptibility and its Prevalence. A.E. Lagarde^{1,2,3}, E. Baumann¹, L. Danis¹, H.S. Stern^{1,2,3}. 1) Ottawa Health Research Institute, Ottawa, Ontario, Canada; 2) Department of Surgery, University of Ottawa; 3) Ottawa Regional Cancer Centre.

Early onset HNPCC is mostly attributed to predisposing mutations in DNA-mismatch repair genes. *MSH2* and *MLH1* are more often implicated than *MSH6*, *MLH3* or *EXO1*. *TGFBR2*, the gene encoding the TGF- β type II receptor has rarely been linked to HNPCC. The proportion of colon cancers attributable to mutations in all these genes is unclear, so that the identification of at-risk individuals remains confined to patients presenting with either very strong family histories or with highly microsatellite unstable (MSI) tumors. We addressed the possibility that some patients may be carriers of mutations in *MSH2*, *MLH1*, *MSH6* or *TGFBR2* that are not as penetrant as in HNPCC. A series of 84 colorectal patients diagnosed in Eastern Ontario were screened for MSI (15 markers) and for mutations in *MSH2/MLH1* (79/84 by SSCP), *MSH6* (38/84 by sequencing) and *TGFBR2* (31/84 by sequencing). Allele-specific PCR assays were developed for relevant sequence variations. Germline mutations, not detected in >90 control subjects, and other than common polymorphisms, were found in 3.8% (*MSH2,MLH1*), 5.2% (*MSH6*) and 2.8% (*TGFBR2*) of tested patients respectively, and overall in 5% of patients younger than 50 years of age. Five mutations were missense, and one was the recurrent a(IVS5, +3)t splice site alteration in *MSH2*. Only this and Met373Ile *TGFBR2* were associated with demonstrated loss of the normal allele in tumors. All were associated with patients having at least one first-degree relative affected by cancer, and all but IVS5 *MSH2* led to low/very low MSI. Both IVS5 *MSH2* and Val878Ala *MSH6* carriers were 2 out of the 6 HNPCC patients present in this series. Furthermore, Gly322Asp *MSH2* heterozygotes accounted for 5.9% of colon cancer patients and 4.9% of controls, but for 16% and 3% of young vs old (>50) patients respectively. After excluding 2 presumed non-penetrant missense mutations, we estimate the carrier frequency in the general population of Ontario to be 1:1400 (*MSH6*), 1:1400 (*TGFBR2*), 1:700 (*MSH2,MLH1*) and 1:280 (*Gly322Asp*).

Two novel *BRCA1* mutations in breast and ovarian cancer patients from Brazil. *J.J. Lourenco, F.R. Vargas, J. Bines, E.M. Santos, C.P. Lasmar, C.H. Costa, E.M. Teixeira, M.C.M Maia, F. Coura, C.H.D. Silva, M.A.M Moreira.* Genetics, Instituto Nacional do Cancer, Rio de Janeiro, RJ, Brazil.

It is well known that 5-10% of all breast cancers are attributable to germline mutations in *BRCA1* or *BRCA2* genes. Identification and characterisation of these mutations are important for genetic counselling and for implementing adequate measures aimed at early diagnosis. DNA sequencing of *BRCA1* and *BRCA2* was carried out in a sample of 40 Brazilian patients with breast and/or ovarian cancer who were selected by our Genetic Counselling Group either because of familial history of breast/ovarian cancer, age at diagnosis or tumour bilaterality. All *BRCA1* and *BRCA2* exons and splicing sites were sequenced. *BRCA1* or *BRCA2* germline mutations were found in 8 patients. Two not previously described mutations were found in *BRCA1*: a transversion (IVS17+2 T>C) in intron 17 at the splicing site and a transition in codon 1,135 (1135C>T; exon 11) resulting in a stop codon (CAG®TAG; GlnStop). Three previously described mutations were also found: (1) a rare *BRCA2* mutation (IVS21+4 A>G) in one Italian patient; (2) *BRCA1* 5382insC in exon 20 in 4 patients; and (3) a 4-nucleotide deletion in *BRCA1* exon 11 in codon 1,111 (3450del4) in one patient. The two novel mutations contribute to the characterisation of the mutational spectrum of *BRCA1* and *BRCA2*.

Analysis of Fanconi Anemia Gene Inactivation in Sporadic Squamous Cell Carcinoma of the Head and Neck. *B. Haider¹, M. Tischkowitz¹, J. Jones², M. McGurk², C.G. Mathew¹, S.V. Hodgson¹.* 1) Division of Medical and Molecular Genetics, Guy's Hospital, London, London, UK; 2) Department of Oral and Maxillofacial Surgery, Guys Hospital, London, UK.

Fanconi Anemia (FA) is a chromosomal breakage syndrome characterised by congenital abnormalities, bone marrow failure and an increased risk of leukemia and some solid tumours, notably squamous cell carcinomas of the head and neck (SCCHN). Given the high incidence of SCCHN in FA homozygotes, we investigated whether inactivation of the FA genes plays a causal role in sporadic SCCHN. Loss of heterozygosity (LOH) analysis was performed on DNA from tumour/normal tissue paired samples from sporadic SCCHN cases using microsatellites flanking the six cloned FA genes (FANCA, C, D2, E, F and G). LOH was found at the FANCD2 locus in 8 of 25 patients (36%) informative for D3S1597 and 2 of 29 patients (7%) informative for D3S1560. LOH was also found at the FANCG locus in 2 out of 31 (6%) patients informative for D9S1874. Quantitative Fluorescent PCR (QF-PCR) was performed on samples showing LOH to assess whether intragenic deletions were present; exons 1,5 and 44 were tested in FANCD2 and exons 1,2,10,14 were tested in FANCG. Two out of the 9 samples that showed LOH at the FANCD2 locus had deletions of all FANCD2 exons tested and both of the FANCG LOH samples had deletions of all FANCG exons tested. Analysis of the methylation status of the CpG island in the promoter region of FANCD2 and FANCG in LOH samples was performed by sodium bisulfite sequencing. None of these samples showed evidence of methylation. Correlation with clinical details showed that patients with FANCD2 LOH in their tumours were on average 10 years younger, there was a lower incidence of heavy smokers and a higher frequency of metastatic disease compared with patients showing no LOH. In summary, these findings suggest that FANCD2 and FANCG may be deleted in some cases of SCCHN. However, there was no evidence to suggest that inactivation of the remaining allele occurred by methylation. These results provide evidence of possible involvement of FANCD2 and FANCG in the etiology of SCCHN.

A 20 year perspective of the International Fanconi Anemia Registry (IFAR). *D.I. Kutler^{1,2}, B. Singh^{1,2}, J. Satagopan², M. Berwick², P.F. Giampietro¹, S.D. Batish¹, A.D. Auerbach¹.* 1) The Rockefeller University, New York, NY; 2) Memorial Sloan-Kettering Cancer Center, New York, NY.

Fanconi anemia (FA) is characterized by a cellular hypersensitivity to DNA crosslinking agents and cancer predisposition in patients. Recent studies demonstrate interactions among *ATM*, *BRCA1*, *BRAC2* (putative *FANCD1* gene) and *FANCD2*, suggesting a function for the FA proteins in DNA repair/recombination, cell cycle control and transcription. The IFAR, a prospectively collected database of FA families registered over the past 20 years (1982-2001), allows us the unique opportunity to analyze the natural history of this rare heterogeneous disease, to perform genotype/phenotype correlations, and to study cancer epidemiology in FA families. We report here on 754 subjects with a diagnosis of FA confirmed by cytogenetic studies at RU. Of these 754 patients, 601 (79.7%) experienced the onset of bone marrow failure (BMF) and 173 (23%) developed a total of 199 malignancies. Of these 199 malignancies, 120 (60%) were hematologic and 79 (40%) were non-hematologic. Twenty-one patients developed more than one neoplasm. The most common solid tumors were squamous cell carcinomas (SCC) of the head and neck, anogenital region and skin. There was no increased risk of SCC in FA patients after hematopoietic stem cell transplant (HSCT) vs. non-transplanted patients. The risk of developing BMF, hematologic and solid neoplasms increased with advancing age, with a 90% 33% and 28% cumulative incidence by 40 years of age, respectively. Univariate analysis revealed significant earlier onset of BMF for group FA-C compared to groups A and G, however there was no significant difference in the time to tumor development between the different groups. Multivariate analysis of overall survival time shows that *FANCC* mutations ($p=0.004$, early hematological onset ($p < 0.0001$)) and HSCT ($p < 0.0001$) define a poor-risk subgroup with significantly worse survival. This study of IFAR patients over a 20-year period enables us to define the natural history of this rare heterogeneous disorder. This information will allow us to better predict outcome and aid clinicians with decisions regarding major therapeutic modalities.

Role of *Cyp17* and *ERb* genes in Hereditary Nonpolyposis Colorectal Cancer. *S.A. Kuismänen*¹, *S. Bala*², *P. Peltomäki*¹. 1) Department of Medical Genetics, 00014 University of Helsinki, Finland; 2) Human Cancer Genetics, Comprehensive Cancer Center, Ohio State University, Columbus, OH 43210.

Two of the most common cancers in the hereditary nonpolyposis colorectal cancer (HNPCC) spectrum are cancer of the colon and of the endometrium. Observations that link the sex hormones in the development of these cancers in the general population prompted us to study the possible modifying roles of the hormone-related *Cyp17* and *ERb* genes. Both of these genes exhibit a nucleotide change that causes variants which may have involvement in the increased risk to cancer. The *Cyp17* gene, which regulates androgen biosynthesis, has a T to C change in the promoter region and gives rise to an A2 variant that may be associated with elevated levels of circulating sex hormones. *ERb*, encoding an estrogen receptor that regulates ovulation as well as being observed to be the primary ER in the colon, has a G to A substitution in exon 8. We analyzed the allele distribution against clinical parameters in a series of 173 affected carriers of 10 MLH1 and 4 MSH2 mutations and 183 mutation negative cases (controls). Individuals carrying an ancestral founding mutation in MLH1 ("mutation 1") constituted the majority of the cases and formed a subgroup with a homogenous background.

The observed genotypes of mutation positive individuals showed some interesting associations relative to age and location of colon cancer in the "mutation 1" group. The variant A2 allele of *Cyp17* (genotype A2/A2) showed significant association with lower age of onset of colon cancer in males (41 vs. 45 years) and an even more striking difference in females (39 vs. 50 years), as compared to genotype A1/A1. Furthermore, the presence of the A2 allele was also associated with distal location of the CRC in females. In *ERb*, homozygosity for the variant allele (A) was found to have a strong association with late onset of colon cancer in "mutation 1" males but not females. We conclude that the *Cyp17* T>C polymorphism may modulate the age at onset and location of colon cancer in HNPCC, especially in the female gender and that *ERb* G>A polymorphism may in turn modulate the age of onset in male HNPCC patients.

Familial prostate cancer in Tasmania. *J.D. McKay¹, J.L. Dickinson¹, D.A. Mackey², M.M. Sale³, D. Shugg¹, M. Ring¹, S. Inglis¹, A. Edwards¹, S. Easta¹*. 1) Menzies Centre for Population Health Research, Uni of Tasmania, Hobart, Tasmania. Australia; 2) Royal Hobart Hosital, Tasmania, Australia; 3) Center for Human Genetics, Wake Forest Uni. School of Medicine, Wiston-Salem, USA.

9% of prostate cancer is thought to be of familial origin. The search for potential susceptibility genes in familial prostate cancer (fPC) has been hindered by the existence of significant genetic heterogeneity, a high phenocopy rate and the late onset of the disease influencing the availability of genetic material in older generations. The Australian island state of Tasmania has a stable population and a high patient participant rate, making an ideal environment to study the aetiology of fPC. From the period of 1978-1998, in excess of 3650 prostate cancer cases have been identified in the Tasmanian cancer registry. By using a novel approach of identification of over-represented names in the Tasmanian cancer registry relative to the phone directory, fPC pedigrees in Tasmania have been identified. Thus far, this technique has identified over 65 families in Tasmania with fPC, with between 2 and 16 affected patients and 6 pedigrees containing more than nine cases. This is therefore, a significant resource for the study of the molecular genetics of fPC and work has commenced to determine the significance of the RNASEL and ELAC2 genes and the known prostate susceptibility loci in the Tasmanian population.

Cancer screening practices following genetic counseling/testing in families with HNPCC. *D. Hadley¹, J. Jenkins², M. DeCarvalho², E. Dimond², I. Kirsch², C.G.S. Palmer³.* 1) NHGRI/NIH, Bethesda, MD; 2) CCR/NCI/NIH, Bethesda, MD; 3) Dept Psychiatry, UCLA.

Little is known about cancer screening practices following genetic counseling and the receipt of genetic test results for cancer susceptibility genes. One goal is to focus cancer-screening in individuals identified at increased risk due to the presence of a familial mutation. A second goal is to reduce cancer-screening to general population levels in those not carrying familial mutations. Data on cancer screening behaviors following the receipt of genetic test results is limited. We report on cancer-screening practices of unaffected individuals receiving results in families with newly identified mutations in Hereditary Non-Polyposis Colorectal Cancer (HNPCC) genes. Individuals in families with an HNPCC mutation were recruited for a study assessing outcomes of genetic counseling/testing. Participants received genetic counseling followed by the offer of gene testing. Those choosing testing received results in a second counseling session. Verbal and written cancer screening recommendations following published guidelines were provided in both sessions. Data on cancer screening practices, psychological variables, attitudes, and beliefs were collected prior to testing and at 6 & 12 months post-test. For each cancer screening behavior, compliance/noncompliance was determined according to the guidelines. Analyses were performed to identify factor(s) associated with compliance. We report on colonoscopy screening behaviors. Of the 60 at-risk individuals with no personal history of cancer, 39 were negative and 21 were positive for an HNPCC mutation. Among the mutation negative, 10.3% were not compliant; half engaged in hyper-vigilance and half engaged in hypo-vigilance. Among the mutation positive, 38.1% were not compliant; 75% engaged in hyper-vigilance and 25% in hypo-vigilance. The data suggest that mutation positive individuals are more likely to be non-compliant for colonoscopy than mutation negative individuals. Furthermore, the predominant form of non-compliance in the mutation positive group is to undergo the procedures more frequently than is generally recommended.

Impact of MC1R variants on melanoma risk in pedigrees with familial and/or multiple melanomas associated with CDKN2A mutations. *S. Majore*¹, *C. Catricala*², *F. Binni*³, *L. Eibenschutz*², *G. Minozzi*³, *P. De Simone*², *A. Amantea*², *P. Grammatico*^{3,1}. 1) Medical Genetics, Az. Osp. S. Camillo-Forlanini, Rome, Italy; 2) S. Gallicano Dermatological Institute, Rome, Italy; 3) Medical Genetics, Experimental Medicine and Pathology Dept., University of Rome La Sapienza, Italy.

The MC1R gene, located in 16q24.3, encodes the melanocytes-stimulating hormone receptor (MSHR), is highly polymorphic in whites (Valverde et al. 1995; Box et al. 1997; Smith et al. 1998) and its variants have been found to be associated with skin phototype. Some recent studies (Van der Velden et al. 2001; Box et al. 2001) seem to evidence a significant association between particular MC1R alleles and an increase of melanoma risk, particularly in families with CDKN2A mutations. CDKN2A mutations predispose subjects to melanoma susceptibility due to an imbalance between functional p16 and cyclin D causing an abnormal cell growth but it is now clear the incomplete penetrance of CDKN2A mutations and the involvement of MC1R polymorphisms in modulating the onset of the disease in subjects belonging to a melanoma-prone family linked to CDKN2A. To better understand the significance of CDKN2A and MC1R variants in pathogenesis of melanoma as well as the weight of the molecular results in genetic counseling of familial and multiple melanomas, we analyzed 90 Italian patients, and several healthy subjects from the same families, by direct sequencing of these two genes. We identified CDKN2A germline mutations in more than 15% of the cases, including the IVS2-105 A/G intronic variant, previously described only in English families (Harland et al. 2001) and some new mutations: a 1bp deletion at nucleotide position 201 (codon 67) (CACGGcGCG) that produces a stop codon (145) by frameshift (Grammatico et al. 2001), a Pro48Thr missense change and a IVS1+2(T-C)/+ mutation interfering with RNA splicing. The analyses conducted on MC1R polymorphisms in patients and in several apparently healthy subjects from families with CDKN2A mutations confirmed the significance of these variants in modulating melanoma onset and age of occurrence.

Penetrance in molecularly proven BRCA1/2 Carriers: Moderate breast cancer risk is increasing in recent cohorts. *E. Levy-Lahad*^{1,2}, *S. Simchoni*^{1,2}, *B. Kaufman*³, *U. Beller*¹, *T. Safra*¹, *P. Renbaum*¹, *R. Catane*³, *A. Lahad*². 1) Medical Genetics, Shaare Zedek Medical Ctr, Jerusalem, Israel; 2) Hebrew University, Jerusalem, Israel; 3) Sheba Medical Center, Tel Aviv, Israel.

Objective: To accurately assess penetrance of the three common BRCA1/2 mutations in the Ashkenazi Jewish (AJ) population, using only molecularly proven carriers. Previous estimates of BRCA1/2 penetrance are extremely variable, ranging from 85% for breast cancer and 56% for ovarian cancer in directly tested high-risk families, to 56% for breast cancer and 16% for ovarian cancer using theoretical models in population-based studies. **Subjects and Methods:** Subjects were BRCA1/2 carriers proven either by direct testing or as obligate carriers per family structure and genotype. Families were ascertained through an affected proband or in our Cancer Genetics clinic. After excluding probands, analysis included 162 non-proband women from 74 families. Breast and ovarian cancer penetrance were analyzed using Kaplan-Meier survival and Cox regression. **Results:** Risk for breast cancer by age 70 was 63% for BRCA1 carriers and 51% for BRCA2 carriers (HR=2.3, p=0.04). Later birth year significantly increased breast cancer risk in BRCA1/BRCA2 carriers (HR=1.04 per year, p=0.009, or HR=1.48 for a 10 yr. difference in birth year). Ovarian cancer risk by age 75 was 63% (SE=9%) for all carriers, not significantly different for BRCA1 or BRCA2 carriers. There was also a trend for increased ovarian cancer risk in later birth cohorts. **Conclusions:** Breast cancer risk for BRCA1/2 carriers in Israel is similar to that estimated for low risk populations, with significantly lower risk for BRCA2 carriers (HR=0.4 vs. BRCA1), whereas ovarian cancer risk is as high as in high-risk families. Cancer risk is increasing significantly in more recent birth cohorts, suggesting a role for environmental and/or reproductive effects.

A *CHEK2* variant with increased breast cancer risk. O. Kilpivaara¹, P. Vahteristo¹, K. Syrjäkoski², H. Eerola^{1,3}, J. Bartkova⁴, K. Aittomäki⁵, P. Heikkilä⁶, J. Bartek⁴, K. Holli⁷, C. Blomqvist^{3,9}, O.-P. Kallioniemi⁸, H. Nevanlinna¹. 1) Dept. of Obstetrics and Gynecology, Helsinki University Central Hospital (HUCH), Finland; 2) Lab. of Cancer Genetics, Inst. of Medical Technology, Tampere University and Tampere University Hospital, Finland; 3) Dept. of Oncology, HUCH, Finland; 4) Inst. of Cancer Biology, Danish Cancer Society, Copenhagen, Denmark; 5) Dept. of Clinical Genetics, HUCH, Finland; 6) Dept. of Pathology, HUCH, Finland; 7) Dept. of Oncology, Tampere University Hospital, Finland; 8) Cancer Genetics Branch, National Human Genome Research Institute, Bethesda, MD; 9) Oncology, Uppsala University Hospital Sweden.

Cell cycle checkpoint kinase 2 (*CHEK2*) is a key mediator of cellular responses to DNA damage. Once activated it phosphorylates various substrates including p53, BRCA1 and CDC25A. Previously, we have identified two *CHEK2* germ line genetic variants in Finnish breast cancer patients and families, I157T in exon 3 and 1100delC in exon 10. The protein truncating mutation 1100delC was previously shown to strongly associate with family history of breast cancer, acting as a low penetrance susceptibility allele. The I157T mutant protein is deficient in binding and phosphorylating p53 and CDC25A. Here, we found that the frequency of I157T was significantly higher among an unselected population-based cohort of 1035 breast cancer patients (77/1035, 7.4%, OR 1.43 95% CI 1.06-1.95) and especially among family history negative patients (53/677, 7.8%, OR 1.52 95% CI 1.07-2.14), as compared to 1885 population controls (5.3%). The frequency in a cohort of 507 familial cases (5.5%) was similar to controls suggesting lower impact of this variant in patients with familial disease. Screening of the whole coding region of the *CHEK2* for novel germ line mutations in 75 breast cancer families revealed four carriers of the 1100delC mutation and five carrying the I157T, however, no additional coding variants were identified. In conclusion, our results indicate that *CHEK2* genetic variants with functional significance are associated with increased breast cancer risk and the I157T is likely to have a contribution to breast cancer at the population level.

Phenotype of *MSH6* versus *MLH1* and *MSH2*: Lower penetrance and higher age of onset . *Y. Hendriks*¹, *A. Wagner*², *H. Vasen*³, *F. Menko*⁴, *A. Stormorken*⁵, *P. Möller*⁵, *M. Genuardi*⁶, *L. Sandkuijl*⁷, *H. van Houwelingen*⁷, *C. Tops*¹, *H. Morreau*⁸, *W. de Leeuw*⁸, *H. Meijers-Heijboer*², *M. Breuning*¹, *R. Fodde*¹, *J. Wijnen*¹, *A. Bröcker-Vriends*¹.
1) Clinical and Human Genetics, Leiden University Medical Center, The Netherlands; 2) Clinical Genetics, Dijkzigt Hospital, Rotterdam; 3) Netherlands Foundation for the Detection of Hereditary Tumours, Leiden; 4) Clinical Genetics, Free University of Amsterdam; 5) Unit of Medical Genetics, University Clinic the Norwegian Radium Hospital, Oslo, Norway; 6) Institute of Medical Genetics, Università Cattolica del Sacro Cuore, Rome, Italy; 7) Medical Statistics, Leiden University Medical Center; 8) Pathology, Leiden University Medical Center.

Background In 50-70% of the families fulfilling the clinical criteria for HNPCC (Hereditary Non Polyposis Colorectal Cancer) a germline mutation is found in *MLH1* or *MSH2*. In 1997 the first germline mutations in *MSH6* were described. Wijnen et al. described 9 pathogenic *MSH6* mutations in 10 families. They found a higher age of onset and lower penetrance compared with *MLH1* and *MSH2*. **Purpose** To further delineate the tumourspectrum and penetrance of pathogenic *MSH6* mutations and to determine a protocol for periodic surveillance. **Methods** We studied 20 families with a mutation in *MSH6*. We performed mutation analysis in 78 clinically affected and 190 at risk carriers. We collected clinical data and performed microsatellite instability analysis and immunohistochemistry on 49 and 41 tumours respectively. **Results** We identified 146 carriers of a pathogenic *MSH6* mutation. We found a lower penetrance for colorectal cancer and a higher penetrance for endometrial cancer when compared with *MLH1* or *MSH2*. We found an average age of onset for both colorectal cancer and endometrial cancer of 55 years. In case of colorectal cancer this is more than 10 years higher than the average age of onset in carriers of a *MLH1* or *MSH2* mutation. **Conclusion** In carriers of a *MLH1* or *MSH2* mutation the current surveillance protocol advises a colonoscopy every 1 to 2 years from the age of 20 to 25 years. We suggest to start with colonoscopies from age 30 in carriers of a *MSH6* mutation.

Some germ-line mutations in mismatch repair genes in HNPCC patients may confer an increased risk for additional germline defects. P. Hutter¹, J. Wijnen², G. Chong³, C. Ray-Berthod¹, I. Thiffault^{3,4}, S. Thibodeau⁵, J. Burn⁶, N. Wong⁴, P. Chappuis⁷, A. de la Chapelle⁸, W. Foulkes⁴. 1) Unit of Genetics, ICHV, Sion, Switzerland; 2) Human and Clinical Genetics, Leiden Univ. Medical Centre, the Netherlands; 3) Diagnostic Medicine, SMBD-Jewish General Hospital, McGill Univ., Montreal, Canada; 4) Human Genetics, McGill Univ., Montreal, Canada; 5) Molecular Genetics, Mayo Clinic, Rochester, MN, USA; 6) Inst. of Human Genetics, Univ. of Newcastle, UK; 7) Div. Oncology/Genetics, Hopital Univ. Geneve, Geneva, Switzerland; 8) Cancer Genetics, Ohio State Univ., OH, USA.

Hereditary non-polyposis colorectal cancer (HNPCC) is thought to account for 2-5% of all colorectal cancer cases. The autosomal dominant syndrome, characterized by colorectal and/or endometrial cancers in the fourth or fifth decade is caused by germline mutations in DNA mismatch repair (MMR) genes. We have identified a number of patients who carry 2 different genetic alterations in MMR genes. Twenty-three probands were found to harbor 2 germline variants in *MLH1*, *MSH2* or *MSH6*. Double heterozygotes corresponded to 27 alterations in *MLH1*, 16 alterations in *MSH2* and 3 alterations in *MSH6*. Noticeably, 8 out of these 46 mutations (17%) involved either codon 618 or 716 of *MLH1*, both of which appear to be among the most frequently altered codons in *MLH1*. We estimate that at least 3% of MMR mutation carriers harbor 2 distinct germline alterations in the above 3 genes.

On further analysis of the *MLH1* haplotype associated with the A/G polymorphism at IVS14-19, it was notable that 25 out of 38 (66%) chromosomes from heterozygotes carried the G haplotype. This haplotype was recently shown to be over-represented on chromosomes bearing *MLH1* mutations, compared with ethnically-matched control groups. These findings may reflect a possible difference in MMR efficiency between A and G haplotypes (Hutter et al. J Med Genet. 39:323-327, 2002).

We hypothesise that some MMR mutations have a weak dominant negative effect which predisposes to other germline defects. This effect may have been overlooked as many centers tend to interrupt their mutation screen once a pathogenic defect is identified.

The Retinoblastoma Gene Mutation Spectrum: Analysis of 240 Independent Mutations. *S.T. May¹, M. Eppstein², M.E. Tang¹, D.W. Yandell¹*. 1) Molecular Diagnostics Lab, Vermont Cancer Center, Burlington, VT; 2) Department of Computer Science, University of Vermont, Burlington, VT.

Tumor suppressor genes provide a unique opportunity to compare in vivo mutation spectra in human genes in both somatic cells and the germline at a single locus. However, reported mutation spectra are often based on compilations of data from multiple studies that have used different detection methods. We report the results of comprehensive mutation analysis in a large series of retinoblastoma cases which identified 240 independent RB gene mutations. The physical characteristics, site and sequence context of these mutations were analysed to define the predominant mechanisms by which spontaneous mutations occur at this locus. We have also compared the physical characteristics of these mutations with clinical and phenotypic features including penetrance and expressivity. Nonsense mutations predominate (74%) with a subset of missense or splicing mutations recurrently associated with low penetrance. 74% of all base substitution mutations were transitions. C to T transitions at CGA arginine codons account for 12 of 16 sites of recurrent mutation and almost 30% of all observed mutations (45% of substitutions); there is a 70-fold bias toward the sense (versus antisense) strand in the occurrence of C to T transition mutations at CpG sites. The ratios of 1) missense to nonsense, and 2) insertion/deletion to base-substitution are different in somatic versus germline mutations. Local sequence context plays an important role in both substitution and insertion/deletion mutation, with regions near potentially stable stem-loop structures and repeated sequences particularly susceptible to mutation. Further analysis of these data is underway to determine whether rules defining the empirical mutation spectrum we have observed can be defined, and might be used to inform strategies to search new or unstudied human sequences for natural polymorphisms and pathogenic mutations.

How BRCA1/2 test results affect risk perception and beliefs among members of Hereditary Breast and Ovarian Cancer (HBOC) Families. *A.M. McInerney-Leo¹, B.B. Biesecker¹, D. Hadley¹, R.G. Kase^{2,3}, T.R. Giambaresi^{2,3}, E. Johnson⁴, C. Lerman⁵, J.P. Struewing².* 1) NHGRI/NIH Bethesda, MD; 2) NCI/NIH Bethesda, MD; 3) Westat Research, Rockville, MD; 4) Johns Hopkins Biostats Center, Baltimore, MD; 5) Univ of Pennsylvania Cancer Center, PA.

Members of HBOC families often have long-held beliefs about their carrier status and the advantages and disadvantages of testing. The extent to which these beliefs affect the decision to undergo testing and the consequences of the receiving results consistent or inconsistent with these beliefs is not known. Within a randomized trial of breast cancer genetic counseling methods, 212 members of 13 HBOC families were offered BRCA1/2 testing for a known family mutation. Risk perception and beliefs about the advantages and disadvantages of testing were assessed at baseline and again at 6-9 months following the receipt of test results, or at the equivalent time for those who declined testing. Comparisons were made between testers and non-testers and as well as between those who tested positive or negative. One hundred eighty-one participants elected to undergo genetic testing (85%) and 47 (26%) were identified as carriers. All individuals who participated believed themselves to be at "a little" to a "much" higher risk for breast cancer as compared to the general population and all felt it was "very likely" or "definite" that they had a mutation, with no difference between testers and non-testers ($p=0.518$). All participants felt that the most important reason to undergo testing was to learn information about their childrens risk. Both groups also felt that the most reason for not wanting testing was worry about losing insurance and non-testers were also worried about confidentiality. Individuals who tested negative had a significant decrease in their perceived risk for breast cancer, ovarian cancer and their chances of carrying an altered gene ($p<0.001$ for all). Those who tested positive had no change in their perceived risks. Conclusion: Perceived risks did not influence the decision to undergo testing. A negative result was followed by a significant decrease in all areas of perceived risk thus changing prior-held beliefs.

Overexpression of the Fanconi anemia protein FANCD2 induces apoptosis. *J. Hejna, D. Bruun, R. Moses.* Dept. of Molecular and Medical Genetics, Oregon Health and Science University, Portland, OR.

Fanconi anemia is a rare, autosomal recessive disease characterized by pancytopenia, and at the cellular level by genomic instability manifested by a hypersensitivity to DNA crosslinking agents. Patients are at elevated risk for certain cancers, including myelodysplasia, acute myelogenous leukemia, and solid tumors. One Fanconi protein, FANCD2, is modified in response to DNA damage and co-localizes with BRCA1 to nuclear foci after DNA damage, implying recruitment to the sites of damaged DNA. We have shown that FANCD2 cDNA constructs under the control of the strong constitutive CMV promoter are toxic to immortalized fibroblasts. By 20 hours after electroporation, most of the transfected cells have either lysed or have rounded up and shrunken, consistent with an apoptotic morphology. We have developed two assays to quantitate the toxicity of constructs carrying the FANCD2 toxic domain. The first is based on co-transfection of subclones of the toxic domain fused to an ECFP reporter, with a second plasmid carrying a non-toxic fluorescent reporter. Toxicity is determined from the ratio of doubly-transfected cells to the total number of fluorescent cells, 24 hours post-transfection. Toxic constructs result in virtually no doubly-transfected cells (only singly-transfected, red-fluorescent cells survive), while non-toxic constructs result in up to 85% co-transfected cells. A second assay, involves subclones of the toxic domain in pIRES constructs, measuring toxicity by a colony-forming assay: since the FANCD2 subclone and the selectable marker for puromycin resistance are co-transcribed, only non-toxic constructs allow colony formation under puromycin selection. Toxicity was determined relative to non-toxic controls. The two assays define the minimal toxic domain to about 85 aa including part of exon 11, all of exon 12, and part of exon 13. Caspase-3 assays also indicate that cell death induced by overexpression of the FANCD2 toxic domain is apoptotic. One possible function of FANCD2 under certain conditions may be to trigger apoptosis.

Multiple primary melanomas in a CDKN2A mutation carrier exposed to ionizing radiation. *S.A. Leachman^{1,2}, C. Hansen^{1,2}, L. Wadge², P. Porter-Gill^{1,2}, S.R. Florell^{1,2}, L. Swinyer¹.* 1) Dermatology, University of Utah Salt Lake City, UT; 2) Huntsman Cancer Institute, University of Utah Salt Lake City, UT.

The development of melanoma involves complex interactions between inherited genetic susceptibility and exposure to environmental risk factors. These interactions are beginning to be explored at the molecular level. Patients with more than five primary melanomas are unusual and can be serendipitous for cellular and molecular investigation. We have investigated a patient with eight primary melanomas in an effort to identify risk factors that may contribute to an exaggerated manifestation of disease. Germline mutations in the gene encoding cyclin dependent kinase inhibitor 2A (CDKN2A, p16, INK4a) are present in 8-15% of patients with multiple primary melanomas and in as many as 75% of patients with 5 or more primary melanomas. However, family members with the CDKN2A mutation do not usually demonstrate multiple primary melanomas and it is unclear why particular individuals suffer an increased risk. Although increased exposure to ultraviolet light is believed to increase penetrance of the CDKN2A gene, the role of additional environmental risk factors in the penetrance of CDKN2A has not been studied. Sequence analysis of CDKN2A in our identified patient revealed a previously reported -34 (G>T) 5' UTR germline mutation that creates an alternative start site and frame shift relative to normal CDKN2A. Cytogenetic evaluation, including fluorescent in situ hybridization, failed to identify additional chromosomal abnormalities in lymphocytes or cultured skin fibroblasts. However, the patient was exposed to ionizing radiation from nuclear testing in Nevada in the 1950s. Radioactive dust deposited preferentially on the right side of her body where six of the eight melanomas arose. Exposure to ionizing radiation in a genetically predisposed individual offers a potential hypothesis to explain the number and distribution of melanomas in this patient. The clinical implications of this potential association would be important to other carriers of p16/CDKN2A mutations because radiotherapy is frequently considered as a treatment option in melanoma patients.

Biallelic Inactivation of BRCA2 in Fanconi Anemia. *N.G. Howlett¹, T. Taniguchi¹, S. Olson², B. Cox², Q. Waisfisz³, C. de Die-Smulders⁴, N. Persky¹, M. Grompe², H. Joenje³, G. Pals³, H. Ikeda⁵, E.A. Fox¹, A.D. D'Andrea¹.* 1) Pediatric Oncology, Dana-Farber Cancer Institute, Boston, MA; 2) Department of Molecular and Medical Genetics and Department of Pediatrics, Oregon Health Sciences University, Portland, OR; 3) Department of Clinical Genetics and Human Genetics, Free University Medical Center, Amsterdam, The Netherlands; 4) Department of Clinical Genetics, Academic Hospital Maastricht, Maastricht, The Netherlands; 5) Department of Pathology, Sapporo Medical University School of Medicine, Sapporo, Japan.

Fanconi Anemia (FA) is an autosomal recessive cancer susceptibility syndrome characterized by multiple congenital anomalies and progressive bone marrow failure. FA patients often develop acute myeloblastic leukemia, but also develop squamous cell carcinomas. FA cells are hypersensitive to crosslinking agents, e.g. mitomycin C (MMC). At least eight distinct complementation groups of FA have been defined, and six FA genes have been cloned (A,C,D2,E,F,G), but the gene(s) corresponding to subtypes B and D1 remains unidentified. Many lines of evidence suggest genetic interactions among the breast cancer susceptibility genes, BRCA1 and BRCA2, and the FA genes. First, assembly of the multisubunit nuclear FA complex (A,C,E,F,G), in response to DNA damage or during S phase of the cell cycle, activates the monoubiquitination of the D2 protein, targeting D2 to BRCA1-containing nuclear foci. Second, BRCA(-/-) tumor cells exhibit MMC hypersensitivity and chromosome instability, similar to FA cells. Third, targeted disruption of the 3' region of the murine *Brca2* gene results in viable mice with an FA-like phenotype. To further investigate the relationship between the BRCA genes and FA, we sequenced BRCA1 and BRCA2 in cells derived from FA-B and FA-D1 patients. While no BRCA1 mutations were detected, biallelic mutations in BRCA2 were observed in the FA-D1 cell lines HSC62 and EUFA423, as well as the FA-B cell line HSC230. BRCA2 western analysis revealed expression of full length BRCA2 protein in HSC62, while only C-terminus truncated BRCA2 proteins were evident in EUFA423 and HSC230.

Localization of genes predisposing to acute leukemia in Down syndrome-A Childrens Oncology Group Study.

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In COG-B957, we are examining the possibility that predisposition to acute leukemia in Down syndrome is the result of increased disomic homozygosity at one or more loci on the non-disjoined chromosome 21 (reduction to homozygosity from heterozygous carrier). If the gene(s) acts as a tumor suppressor, a somatic event would be expected to unmask recessive alleles on the non-disjoined chromosome 21 (Am J Med Genet; 59:174-181). Microsatellite loci spanning the entire length chromosome 21 (n=50) were genotyped at diagnosis and remission and compared with parental genotypes for 22 patients (13 with AML, 9 with ALL). Linkage analysis of markers from D21S369, near the centromere, to D21D49, the most telomeric locus, revealed two genetic intervals (I and II) with high frequencies of disomic homozygosity. Within interval I, a locus at ~11 million base pairs (Mb) in the sequence of chromosome 21 had a maximum 2-point lod score of 4.82 for patients with at least one reduced locus. Z_{\max} was 5.30 at ~12.5Mb, when all 22 patients were analyzed. In interval II, the maximum lodscore was 3.31 between 25 -28 Mb, which includes the region containing AML1, the gene mutated in Familial Platelet Disorder. When all patients were considered, $Z_{\max} = 6.92$ for interval II. Somatic mutations confined to the diagnostic marrow were observed at 12.5 Mb in 2 individuals on the normally disjoined chromosomes, consistent with a possible second hit at these loci. Loss of heterozygosity (LOH) was evident for markers in 4 patients in the 11-13 Mb interval, as well as the 28-30 Mb (n=3) and 17-18 Mb (n=4) intervals. Three patients exhibited LOH for markers in 2 different intervals. Thus, at least 2 different genes on chromosome 21 appear to contribute to the predisposition to leukemia in Down syndrome. Linked somatic mutations suggest the possibility that a tumor suppressor gene in interval I may be inactivated. Potential gene candidates in interval I for sequence analysis include those involved in cell proliferation (BTG3), signal transduction (Metrin A), a tyrosine kinase (SAMS1), and chaperonin (STCH), and in interval II, a ser/thr kinase (HUNK), and a cell cycle regulator (TIAM1).

Putative common origin of two *MLH1* mutations in Quebec hereditary non-polyposis colorectal cancer (HNPCC) families. I. Thiffault¹, G. Chong^{1,2,5}, N. Wong¹, V. Marcus³, E. MacNamara^{2,3,5}, P. Radice⁶, P. Hutter⁷, W. Foulkes^{1,4}. 1) Department of Human Genetics; 2) Department of Medicine; 3) Department of Pathology; 4) Program in Cancer Genetics, McGill University, Montreal, QC, Canada; 5) Department of Diagnostic Medicine, SMBD-Jewish General Hospital, Montreal, QC, Canada; 6) Department of Experimental Oncology, Istituto Nazionale Tumori, Milano, Italy; 7) Unit of Genetics, Institut Central des Hopitaux Valaisans, Sion, Switzerland.

Hereditary non-polyposis colorectal cancer (HNPCC) is one of the most common inherited cancers accounting for 3-5% of all cases of colorectal cancer. In most HNPCC families, the disease is caused by a germline mutation in *MLH1* or *MSH2*. In some populations, founder mutations appear to explain a substantial fraction of HNPCC. Founder mutations in *MLH1* have been identified in Finland and Switzerland. A germline mutation that may also be a founder mutation (1.8-kb deletion involving exon 11 of *MLH1*) has also been reported in two HNPCC kindreds in China. We report here the identification and preliminary characterization of two putative *MLH1* founder mutations. The mutation *MLH1*:1831 delAT was shown to segregate in two Quebec families of Italian origin who fulfilled the Amsterdam criteria for HNPCC. Haplotype analysis using 5 intragenic micro-satellite/SNP markers spanning *MLH1* on chromosome 3 showed that these two unrelated families share an identical haplotype. By contrast, two other Italian kindreds whose affected members carry *MLH1*:IVS6+3 A>G did not share a common haplotype, suggesting that the latter mutation has arisen independently. The second probable founder mutation involves a deletion of the whole of exon 13 from the cDNA of *MLH1* in several affected members of two unrelated French Canadian kindreds. This out-of-frame deletion results in a premature stop codon 19 bp downstream. The mutation was shown to segregate on a common haplotype in both families, supporting the notion that this is a founder mutation. These two mutations are the first putative founder *MLH1* mutations to be identified in North American HNPCC kindreds.

Germline mutations in the PTCH gene in Italian Gorlin Syndrome patients. *L. Pastorino¹, R. Cusano¹, F. Faravelli², M. Barile¹, P.L. Santi¹, L. Lo Muzio³, A. Tel⁴, G. Bianchi-Scarra¹.* 1) Dept Oncology Biol. Genetics, Univ. of Genova, Genova, Italy; 2) EO Ospedali Galliera, Genova, Italy; 3) Institute of Dental Sciences, Univ. of Ancona, Ancona, Italy; 4) Ospedale S.Martino, Genova, Italy.

Gorlin Syndrome (GS), alternatively called Nevoid Basal Cell Carcinoma Syndrome, is a rare autosomal dominant disorder. GS is characterized by predisposition to basal cell carcinomas (BCC) of the skin, medulloblastomas and other tumors. A variety of malformations is another feature of the syndrome including pits of the palms and soles, odontogenic keratocysts and skeletal alterations. GS is noted for its interfamilial and intrafamilial clinical variability, and no evident genotype-phenotype correlation has been established. The disease is known to result from mutations in the PTCH gene located at chromosome 9q22.3, which causes loss of function in the patched protein. In order to verify the involvement of PTCH among Italian patients we provide a molecular characterization of 5 unrelated Italian probands who met the criteria for GS (keratocysts of the jaw, calcification of the falx cerebri and multiple BCCs), and 7 patients with multiple BCCs presenting at least 3 BCCs before 50 years or 1 before 20 years and no other major criteria. Screening was performed using combined standard SSCP and DHPLC analysis (WAVE DNA fragment analysis system, Transgenomic, Santa Clara, CA). Sequencing of the variants revealed 5 different mutations, 2 missense (L479R, T1194S), and 3 non-sense (2011_2012insC, 2535_2536insG, 2577_2583delTGACAGT), in the 5 GS cases. With the exception of 2011_2012insC, none of these mutations have been previously described. Although none of the 7 BCC patients were found to be mutated, some showed variations of uncertain significance in the introns and exons of the gene. The importance of genetic analysis in Italian GS patients lies in the possibility to provide more accurate pre- and post-natal diagnosis and implement strategies for prevention of UV- and radiation therapy-induced BCC.

IGF-1 and insulin-induced expression of HIF-1 through activation of PI3K/Akt signaling in ovarian cancer cell lines. *H.D. Skinner, J.Z. Zheng, N. Gao, X.S. Zhong, L.A. Lopez, B.H. Jiang.* MBR Cancer Center, Dept. of Microbiology, Immunology and Cell Biology, WVU School of Medicine, Morgantown, WV.

Ovarian cancer continues to be one of the most prevalent female cancers. The gene coding for p110 α , the catalytic subunit of phosphatidylinositol 3-kinase (PI3K), is frequently amplified in ovarian carcinoma. Hypoxia-inducible factor 1 (HIF-1) is a basic helix-loop-helix transcription factor that is induced by hypoxia, growth factors and oncogenes. HIF-1 plays an important role in angiogenesis and tumorigenesis. Insulin and IGF-1 have been shown to be mitogenic in a number of human carcinomas. In this study we determined whether activation of PI3K/Akt mediated the expression of HIF-1 in several ovarian carcinoma cell lines. We demonstrated that increased expression of HIF-1 α is mediated through PI3K/Akt. We also showed that IGF-1 and insulin specifically induced HIF-1 α , but not HIF-1 β protein. The induction of HIF-1 α expression by insulin and IGF-1 was inhibited by the addition of the PI3K inhibitors LY294002 and Wortaminin, which indicates that the induction of HIF-1 α expression was mediated by PI3K signaling. These results suggest that HIF-1 may play an important role in ovarian carcinoma tumor progression due either to the amplification of PI3K or the increased expression of growth factors.

Investigation of candidate genes located within the chromosome 6q24.1-25.2 cytogenetic location in adenoid cystic carcinoma of the salivary gland. *S. Rutherford*¹, *C.A. Rumpel*¹, *H.F. Frierson Jr*¹, *C.A. Moskaluk*². 1) Dept of Pathology, University of Virginia, Charlottesville, VA; 2) Depts of Pathology and Biochemistry and Molecular Genetics, University of Virginia, Charlottesville, VA.

Deletion of chromosome 6q has been described in adenoid cystic carcinoma (ACC) of the salivary gland, consistent with targeted inactivation of a tumor suppressor locus. In order to identify possible tumor suppressor genes, we previously conducted comparative genomic hybridization (CGH) and loss of heterozygosity (LOH) analyses to characterize regions of genetic deletion on chromosome 6 in ACC. The results from these ACC studies show the greatest loss occurring within the 6q24.1-6q25.2 cytogenetic location which also harbors the tumor suppressor genes *pleomorphic adenoma of the salivary gland gene like 1 (PLAGL1/LOT1/ZAC)* and *large tumor suppressor gene-1 (LATS1)*. We therefore evaluated both *PLAGL1* and *LATS1* as possible candidate tumor suppressor genes in ACC. In this study, we performed a sequencing based mutation analysis for *PLAGL1* and *LATS1* in 5 ACC samples with monoallelic deletion in this area. In each of the 5 tumor samples, DNA was extracted from microdissected paraffin embedded tumor tissue. All exons of *PLAGL1* and *LATS1* were PCR amplified and sequenced using the Applied Biosystems 377 Prism DNA Sequencer that employs BigDye terminator chemistry with Taq DNA polymerase. No mutations were identified in *PLAGL1* and *LATS1* in the 5 ACC samples tested, suggesting that these genes are not common targets of biallelic genetic inactivation. Further studies using gene transfer and gene expression analysis are required in order to determine if these tumor suppressor genes play a role in ACC progression.

Germline mutations and uncertain variants of the BRCA1 and BRCA2 genes in a hospital-based series of breast cancer patients

. R.B. van der Luijt¹, R.P.M. Jansen¹, P.H.A. van Zon¹, M. Oostra¹, N. Smakman¹, V.C.M. Kuck-Koo², C.C. Wárlám-Rodenhuis³, M.G.E.M. Ausems¹. 1) Department of Biomedical Genetics, University Medical Center Utrecht, Utrecht, The Netherlands; 2) Department of Epidemiology, Comprehensive Cancer Center Utrecht, Utrecht, The Netherlands; 3) Department of Radiotherapy, University Medical Center Utrecht, Utrecht, The Netherlands.

Germline mutations in either of two tumor suppressor genes, BRCA1 and BRCA2, predispose to breast and/or ovarian cancer. The study presented here is part of a prospective study on genetic risk factors in 1,000 breast cancer patients receiving adjuvant radiotherapy for their treatment. We performed mutation analysis of the BRCA1 and BRCA2 genes in 137 patients fulfilling the selection criteria for referral to the Family Cancer Clinic of our hospital. A mutation screening protocol aimed at the analysis of regions of both genes containing recurrent deleterious mutations was used. This partial analysis of BRCA1 and BRCA2 employed the protein truncation test (exon 11 of BRCA1; exon 10 and 11 of BRCA2), denaturing gradient gel electrophoresis (DGGE; exon 2 and 20 of BRCA1), and a PCR-based deletion test (exon 13 and 22 of BRCA1). In addition, the entire coding region of BRCA1 and BRCA2 was analyzed by DGGE in a subset (n=37) of the patients. In 18 patients, germline alterations of the BRCA1 and BRCA2 genes were identified. Nine of the sequence alterations are clear deleterious mutations. As expected, these include several known founder mutations present in the Dutch population. The other nine genetic changes were variants of uncertain clinical significance. They included aminoacid changes as well as single base substitutions in intronic sequences. The prevalence of BRCA1 and BRCA2 mutations (7%) is low compared with breast cancer patients referred to our department outside this study (16%), but is in accordance with mutation prevalences observed in other hospital-based series of breast cancer patients. Correlations between risk factors and the mutations detected are currently being investigated.

Cancer Risk in NBS Heterozygotes from the Czech Republic. *K. Sperling¹, E. Seemanova², R. Varon¹, P. Jarolim², J. Pelz¹*. 1) Institute of Human Genetics, Charite, Humboldt University, Berlin, Germany; 2) Dept. of Medical Genetics, Charles University, Prague, Czech Republic.

The chromosomal instability disorder Nijmegen Breakage Syndrome (NBS) is associated with extreme susceptibility to lymphoid malignancies due to a defect in DNA double strand break repair. Based on familial data it has been suggested that NBS heterozygotes have an elevated cancer risk (Seemanova, E., 1990). Here, we present 1. the results of a cohort study (index-test method) based on the histories of 251 persons from 21 Czech pedigrees. Each individual was personally interviewed and detailed information was collected. The items of relevance comprised: reproductive history, X-ray history, working place history, former and current health status and possible confounding factors. Of the 251 persons analysed for the 657del5 founder mutation, we found 108 heterozygotes and 143 normal homozygotes with almost identical mean age. There were 13 cases of cancer among the heterozygous subgroup, as opposed to 3 cancer cases found among the normal homozygotes ($p < 0.01$). Amongst others, the heterozygotes developed the following type of tumours: carcinoma coli, - ventriculi and - mammae, myeloma multiplex and lymphoma malignum. 2. In addition, we have interviewed and genotyped 60 grandparents from 16 families (ascertainment is almost complete). Ten out of the 30 NBS heterozygotes but only one out of the 30 normal homozygotes developed cancer. Both groups showed no difference in age distribution and socio-economical status. These studies confirm the earlier suggestion that NBS gene carriers have an elevated cancer risk.

Atypical Li-Fraumeni-like syndrome due to a novel exon 6 TP53 germline mutation. *M.W.G. Ruijs¹, S. Verhoef¹, G. Wigbout¹, A. Floore¹, R. Pruntel¹, L.J. van 't Veer¹, F.H. Menko².* 1) Family Cancer Clinic, The Netherlands Cancer Institute, Amsterdam, The Netherlands; 2) Family Cancer Clinic, Department of Clinical Genetics and Human Genetics, VU University Medical Center, Amsterdam, The Netherlands.

Li-Fraumeni syndrome (LFS) and Li-Fraumeni-like syndrome (LFL) are autosomal dominant cancer predisposition syndromes with high penetrance, in which susceptible individuals may develop a wide variety of early-onset malignancies, including sarcoma, brain tumor and breast cancer. In many of the LFS/LFL families, germline mutations in the TP53 gene have been identified; the majority missense mutations. We present an extended Dutch kindred with clustering of a variety of cancers, in which a TP53 mutation was found in exon 6, Arg213Gln. The tumors observed in 7 proven or obligate mutation carriers are atypical with respect to tumor type and age at diagnosis. They include breast cancer in three patients (mean age at diagnosis 60 yrs), renal cell carcinoma (1 patient, 48yrs), melanoma (1 patient, 46yrs), oesophagus-cardia carcinoma (1 patient, 71yrs) and mesothelioma (1 patient, with asbest exposure, 55 yrs). The missense mutation found in this family has not previously been identified as a germline mutation in LFS or LFL families. Several observations suggest that this novel missense mutation is the underlying causative factor, including its identification as a somatic mutation in different tumors, e.g. bladder, breast, and colon, the occurrence of other codon 213 germline mutations associated with LFS/LFL and co-segregation of the mutation with the disease in this family. In a functional yeast-based assay, with a p53 inducible promotor, it could be shown that the mutated allele lacks biological transcriptional activity. Ongoing studies include characterization of genetic alterations in tumor tissue and screening of 250 healthy controls for this TP53 Arg213Gln mutation. In summary, we observed an atypical spectrum of tumors with an unusual age of onset in an extended LFL kindred with a novel TP53 mutation. Our observations contribute to gaining insight into genotype-phenotype correlation of TP53 germline mutations.

Spectrum of Mutations Detected in the RET Proto-oncogene Associated with Multiple Endocrine Neoplasia Type

2. *W. Sun¹, A. Buller¹, R. Fenwick², F. Hantash¹, S. Huang¹, M. Peng¹, J. Redman¹, C. Strom¹.* 1) Molec Gen Dept, Nichols Inst, Quest Diagnostics Inc, San Juan Capistrano, CA; 2) Molec Endocrine Dept, Nichols Inst, Quest Diagnostics Inc, San Juan Capistrano, CA.

Multiple endocrine neoplasia type 2 (MEN2) is an autosomal dominant disease characterized by medullary thyroid cancer, pheochromocytoma, parathyroid adenoma and mucosal neuromas. Detection of disease-causing mutations in the RET proto-oncogene confirms the clinical diagnosis of MEN2 and familial medullary thyroid cancer (FMTC). Moreover, detection of such mutations in at-risk family members allows pre-symptomatic diagnosis and treatment, thereby reducing morbidity and mortality. In this study, we determined the frequency of RET mutations detected in 1993 consecutive samples (3986 alleles) received at Quest Diagnostics Nichols Institute for RET proto-oncogene mutation testing. Using PCR amplification and automated DNA sequencing of exons 10, 11, 13, 14, and 16, we detected 254 mutant alleles. After eliminating all but the probands of the affected kindreds, the following mutation frequencies were observed: 24.7% for C609Y, 16.4% for C634R, and 12.8% for V804M. Five novel missense mutations and one novel trinucleotide deletion were also detected. C609Y, the most common mutant allele in our samples, occurred at a greater frequency than that reported by others. On the other hand, mutations at codon C634 represent only 29.2% of all mutations detected in our study, less than what would be expected based on previous reports. For example, Muligan et al (1995) found codon C634 mutations accounted for 85% of RET mutations in MEN2A patients (or over 50% of MEN patients). Nevertheless, C634 is still a mutation hotspot in our test population, along with C609, C618, C620, and V804. Nucleotide substitutions altering amino acid coding at these locations collectively accounted for over 83% of disease-causing mutations in our test population. The unexpected high prevalence of mutations outside of C634 in our test population may suggest under ascertainment in previous studies.

Identification of cyclin D1 (CCND1) and other novel targets for the VHL tumour suppressor gene (TSG) and investigation of CCND1 genotype as a modifier in VHL disease. *M. Zatyka¹, N.F. da Silva¹, S.C. Clifford¹, M.R. Morris¹, M.S. Wiesener², K.U. Eckardt², R.S. Houlston³, F.M. Richards¹, F. Latif¹, E.R. Maher¹.* 1) Med & Molec Genetics, University of Birmingham, UK; 2) Charit, Humboldt-University, Berlin, Germany; 3) Section of Cancer Genetics, Institute of Cancer Research, Sutton, UK.

Germline mutations in the VHL TSG are associated with a high risk of retinal and cerebellar hemangioblastomas (HABs), renal cell carcinoma (RCC) and pheochromocytoma. pVHL has a critical role in regulating proteosomal degradation of the HIF transcription factor and VHL inactivation results in overexpression of a wide range of hypoxia-inducible mRNAs including VEGF. In order to identify novel pVHL target genes we investigated the effect of wild type pVHL on the expression of 588 cancer related genes in two VHL-defective RCC cell lines. Expression array analysis identified 9 genes which demonstrated a >2-fold decrease in expression in both RCC cell lines following restoration of WT pVHL. Three of the 9 genes (VEGF, PAI-1 and LRP1) had previously been reported as pVHL targets and are known to be hypoxia-inducible. In addition 6 novel targets were detected: cyclin D1 (CCND1), cell division protein kinase 6 (CDK6), collagen VIII alpha 1 subunit (COL8A1), CD59 glycoprotein precursor, integrin beta 8 (ITGB8), interleukin 6 precursor (IL-6, interferon beta 2). We found no evidence that CCND1, CDK6, CD59 and ITGB8 expression was influenced by hypoxia suggesting that pVHL downregulates these targets by a HIF-independent mechanism. Other studies have suggested that (a) genetic modifiers influence the phenotypic expression of VHL disease and (b) polymorphic variation at a CCND1 codon 242 A/G SNP may influence cancer susceptibility or prognosis in some situations. We therefore analysed the relationship between CCND1 genotype and phenotypic expression of VHL disease. There was an association between the G allele and multiple retinal angiomas ($p=0.04$) and risk of CNS haemangioblastomas ($p=0.05$). These findings suggest that polymorphic variation at pVHL target genes may influence phenotypic expression of VHL disease.

Family characteristics associated with nonreferral of relatives for HNPCC genetic counseling and testing. *S.K. Peterson¹, B.G. Watts¹, P.M. Lynch¹, S.W. Vernon², S. Kapoor¹, E.R. Gritz¹.* 1) UT MD Anderson Cancer Ctr, Houston, TX; 2) UT School of Public Hlth, Houston, TX.

Despite potential benefits of cancer genetic counseling and testing (GCT), uptake has been lower than expected. Because individuals act as gatekeepers in disseminating genetic information to their relatives, willingness to refer one's relatives for GCT may affect uptake of these services. Using pedigree data, we evaluated characteristics associated with at-risk relatives who were not referred for HNPCC GCT services that were offered free as part of a research study. Of 35 HNPCC mutation-positive probands, 33 chose to receive their results. After results disclosure, probands were invited to refer their at-risk relatives to the study. When we received permission to contact probands' at-risk relatives, GCT was offered using a standard method. As additional carriers were identified in the families, GCT was subsequently offered to their at-risk relatives in the same manner. Of 471 adult relatives who were at 25% or 50% risk of carrying a known mutation and were eligible for GCT, only 52% (n=247) were referred for GCT by probands or other mutation-positive family members. Two probands (with n=23 total eligible relatives) referred no relatives for GCT. In multivariate analysis, at-risk relatives were less likely to be referred if they had no children. In non-White families (n=4 families), men were less likely to be referred. In White families (n=29 families), relatives were less likely to be referred if the family member relating them to the mutation carrier was deceased or if they were in the oldest living generation on the pedigree. Relatives' personal cancer history or mutation risk were not associated with referral for GCT. Data from a companion qualitative study on family communication about HNPCC also support these findings; namely, that probands and other mutation-positive family members may be less likely to take an active role in referring more distant at-risk relatives for GCT, particularly if intervening relatives are deceased. Future research should evaluate strategies that facilitate greater diffusion of HNPCC GCT services through extended high-risk families.

Novel and founder mutations in mismatch repair genes in HNPCC families from Newfoundland. *M.O. Woods*^{1,2}, *J.S. Green*², *J.D. Robb*³, *A. Pollett*¹, *B. Younghusband*¹, *P.S. Parfrey*⁴, *J.R. McLaughlin*⁵, *B. Bapat*¹. 1) Dept of Lab Medicine & Pathobiology, University of Toronto, Toronto, ON, Canada; 2) Dept of Genetics, Memorial University of Newfoundland, St. John's, NF, Canada; 3) Dept of Pathology, Memorial University of Newfoundland, St. John's, NF, Canada; 4) Dept of Nephrology, Memorial University of Newfoundland, St. John's, NF, Canada; 5) Dept of Public Health Sciences, University of Toronto, Toronto, ON, Canada.

Hereditary non-polyposis colorectal cancer (HNPCC) is the most common heritable form of colon cancer. Mutations in five known susceptibility genes involved in mismatch repair (MMR) have been identified in causing HNPCC. Alterations in two, *MLH1* and *MSH2*, make up approximately 90% of all mutations causing HNPCC. The island of Newfoundland has a relatively homogenous population, composed of multiple genetic isolates which have been valuable in genetic research. Due to the population structure of the island, founder mutations are anticipated. A cohort of 27 high-risk Newfoundland HNPCC families has been collected in order to determine their putative genetic defect, assess their cumulative cancer risk and evaluate genotype-phenotype correlations. Of the 27 kindreds, 15 meet Amsterdam criteria I (AC1) and 12 AC2. Of the 15 AC1 families, individuals in 13 have multiple primary cancers (MPC). For the 12 AC2 kindreds, individuals in 9 have MPC.

Automated sequencing of *MLH1* and *MSH2* was used for initial screening of sequence alterations. When available, DNA from tumours was analyzed for microsatellite instability, a hallmark of MMR involvement. All tumours also underwent immunohistochemical analysis to determine MLH1 and MSH2 expression. Southern blot analysis was employed to identify large genomic deletions. Only one family had an alteration in *MLH1* upon sequencing. This putative mutation, V94E, is a novel substitution. A novel mutation, the deletion of exon 8, was identified in *MSH2* and was shown to segregate in two unrelated families. Affected females in these families had an increased predilection for endometrial cancer. Additional HNPCC-like Newfoundland families are being tested for other MMR defects, including *MSH6*.

Empiric risk of prostate cancer for relatives of a prostate cancer patient. *M.P.A. Zeegers¹, A. Jellema¹, H. Ostrer².*
1) Maastricht University, Netherlands; 2) New York University School of Medicine, New York.

Although narrative reviews have concluded that there is strong support for familial clustering of prostate cancer, the association has never been quantified systematically in reviews. The purpose of this meta-analysis was to summarize and quantify the recurrence risk ratio with emphasis on the degree of relatedness, the relationship of the family member, the number of affected family members and the age at diagnosis. The authors included 32 population-based studies and calculated summary recurrence risk ratios (SI) by random effects meta-regression analyses. They also evaluated changes in summary estimates according to differences in study methodology. SI was 2.46 (95% CI: 2.14-2.82) for first-degree family members. The SI appeared to be higher for men with an affected brother (3.28, 95% CI: 2.84-3.78) than for men with an affected father (2.18, 95% CI: 1.89-2.51). Whereas the recurrence risk ratio for men with second-degree relatives with prostate cancer was only slightly elevated (1.68, 95% CI: 1.07-2.64). The nature of this familial clustering is such that SI rises with decreasing age of the patient and family members, with increasing genetic relatedness of the affected relative and with an increase in the number of individuals affected within the family. No significant differences were observed in studies that analysed familial recurrence risks based on ethnicity. These studies demonstrate consistently that family history is a significant risk factor for developing prostate cancer. This meta analysis provides precise quantitative estimates that can be used for providing genetic counselling to the male family members of men with prostate cancer.

Origins of neurofibromas in NF1. *T. Tucker, C.J. Brown, J.M. Friedman.* Medical Genetics, University of British Columbia, Vancouver, B.C., Canada.

Neurofibromas are benign tumours arising from peripheral nerves. These tumours are comprised largely of Schwann cells but may contain numerous fibroblasts, perineural cells, endothelial cells and mast cells. Neurofibromas are generally assumed to be neoplasms, but there is evidence that at least some neurofibromas are actually dysplastic or hyperplastic lesions. If neurofibromas are true neoplasms, they would be expected to arise clonally by a "two-hit" mechanism, with one inherited and one acquired mutation of NF1 the locus. On the other hand, if some neurofibromas arise by hyperplasia or dysplasia as a result of NF1 haploinsufficiency, they would not be expected to be clonal or to exhibit NF1 loss of heterozygosity. The purpose of this study is to determine if clonal proliferation of the Schwann cells is necessary and sufficient for the development of discrete dermal neurofibromas in patients with NF1 and if these cells exhibit loss of expression of neurofibromin, the protein product of the NF1 gene. Schwann cells and fibroblasts in discrete dermal neurofibromas from women with NF1 are identified using appropriate membrane markers. Following cellular identification, slides are stained with neurofibromin, to identify Schwann cells and fibroblasts expressing the protein. Schwann cells and fibroblasts expressing neurofibromin and those that lack neurofibromin are isolated using laser microbeam microdissection and laser pressure catapulting and tested for clonality using X-linked polymorphic markers. Fibroblasts are used as a negative control because any skewing of their X-inactivation pattern is likely to be a result of small sample size. Almost all people with NF1 develop multiple discrete dermal neurofibromas, and the number of tumours usually increases with age. Better understanding of the pathogenesis of these benign but ubiquitously tumours may provide insights into development of more severe tumours that also occur in people with NF1.

The genetics of breast cancer in Newfoundland. *T.-L. Young^{1,2}, J.S. Green², S. Snook¹, M. Connolly-Wilson², M. Crowley², K. Laing², K. Dalikashvili¹, R.K. Kim¹, M.C. King¹.* 1) Medical Genetics, University of Washington, Seattle, WA; 2) Memorial University of Newfoundland.

A well-recognized problem associated with the broad mutation spectrum of breast cancer mutations is that information critical to implementation of screening programs, like the age of onset of breast/ovarian cancer, spectrum of associated cancers and the lifetime risk of developing cancer are difficult to assess because the number of cases per mutation is low. Newfoundland, a genetically isolated population of mostly English and Irish extraction, is characterized by large families with a traditional lifestyle and close family ties. Founder mutations have been identified in several cancer syndromes in this population. We have collected DNA samples from women with breast and/or ovarian cancer in 150 Newfoundland families with a history of breast/ovarian cancer. We have completed conventional screening (SSCP, PTT, sequencing) in 90 of these families and identify 7 probands with mutations in BRCA1 and 5 probands with mutations in BRCA2. We have also identified 4 women with the 1100delC mutation in CHK2. We will report on the occurrence of breast cancer, associated cancers, and age-related penetrance in these extended families.

Molecular genetic analysis of archival ovarian tumors confirm probable deleterious nature of an in-frame 15 bp deletion (8457del15) in *BRCA2*. *J.N. Weitzel, S.L. Martinez, J. Herzog.* Clinical Cancer Genetics, City of Hope Cancer Center, Duarte, CA.

Mutations in *BRCA1* or *BRCA2* account for the majority of families with HBOC syndrome. Most clearly deleterious mutations are small insertions or deletions, causing a protein-truncating frame shift. However, minor alterations such as missense mutations or small in-frame deletions in the coding regions of *BRCA1* and *BRCA2* have been reported and are a clinical dilemma because of their classification as variants of unknown significance. It has been suggested that somatic allelic deletion of the wild-type allele in the tumor sample, as detected by loss of heterozygosity (LOH) analysis can be used to determine if these mutations are likely to be deleterious. *BRCA* gene sequencing in a 50-year-old ovarian cancer patient with a family history of breast and ovarian cancer revealed a 15bp in-frame deletion in exon 18 of *BRCA2* (8457del15), classified as a variant of unknown significance. Her deceased mother was diagnosed with breast cancer at 57 and ovarian cancer at 62. Alignment of *BRCA2* amino acid (aa) sequences from five species indicated significant evolutionary conservation (39/50 aa identity in the region, including all five aa in the deletion). To investigate whether the mutation tracked with disease in the family we extracted DNA from paraffin-embedded tumor tissues from the proband and her deceased mother. Primers flanking the 15 bp deletion locus were used to PCR amplify the respective segment of exon 18 from blood DNA for the proband, two unaffected siblings, one offspring and from the tumor blocks. Easily discerned on an 8% acrylamide gel, the mutated allele was detected in both the proband and her mother. However, the unaffected siblings and the daughter were all negative for the mutation. In addition, there was clear LOH of the wild-type allele in both tumors. Analysis of six markers along chromosome 13 (D13S742, D13S218, D13S171, D13S267, D13S263, and D13S248, listed in physical map order) revealed LOH at all informative markers in both cases. Coupled with the data on evolutionary conservation we conclude that the *BRCA2* 8457del15 mutation is highly likely to be deleterious.

Analysis of MLH1 promoter hypermethylation in MSI versus MSS tumors arising in patients with confirmed HNPCC or familial colorectal cancer. V. Pethe^{1,3}, A. Saka^{1,3}, A. Pollett¹, M. Aaronson², H. Rothenmund², M. Redston^{1,3}, S. Gallinger^{2,3}, B. Bapat^{1,3}. 1) Pathology, Mount Sinai Hospital, Toronto, ON, Canada; 2) Surgery, Mount Sinai Hospital, Toronto, ON, Canada; 3) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada.

Hereditary nonpolyposis colorectal cancer (HNPCC) is recognized by a dominant inheritance pattern of early-onset CRC and an increased frequency of certain extra-colonic cancers. Defects in mismatch repair (MMR) pathway cause HNPCC and also account for ~15% of sporadic CRC. MMR deficient tumors exhibit a mutator phenotype known as microsatellite instability (MSI). Among MMR genes, inactivation of MLH1 via promoter hypermethylation is shown to be the principal mechanism of MMR deficiency in MSI + sporadic CRC, however, its specific contribution to inherited CRC is largely unknown. We investigated MLH1 hypermethylation in CRC tumors from individuals with confirmed HNPCC (N=20; 10 each with germline MSH2 and MLH1 mutations) or familial CRC (N=28). Families were identified through population-based (OFCCR) or hospital-based (FGICR) CRC registries. Fifty-nine tumors (43 MSI+, 16 MSS) from 48 patients were investigated for MLH1 hypermethylation by methylation-specific PCR (MSP) [nts: 721-597 upstream from transcription start site, GenBank # U83845), MLH1/MSH2 protein expression by IHC and germline MLH1/MSH2 mutations by sequence analysis. 13/43 (30%) MSI+ and 6/16 (37%) MSS tumors were methylated for MLH1. Interestingly, 4/20 (20%) HNPCC patients with known germline MMR mutation (2 MSH2 and 2 MLH1) exhibited MLH1 hypermethylation. Of 13 MSI+ and methylated tumors, 11 were MLH1 immunodeficient indicative of biallelic inactivation, while none of the methylated MSS tumors showed absence of MLH1 protein expression, suggesting either partial methylation or monoallelic inactivation. Our results suggest that MLH1 promoter hypermethylation occurs at a comparable frequency among individuals with either confirmed or suspected HNPCC and may contribute to a distinct pathway of colorectal tumorigenesis.

Association of the C677T and A1298C polymorphisms in the MTHFR gene with breast cancer risk in Turkish women. A. SAZCI¹, E. ERGUL¹, Z. UTKAN², Z. CANTURK², M. MUSLUMANOGLU³, A. IGCI³ and Cancer Research Study Group. 1) Department of Medical Biology & Genetics, Kocaeli University, Kocaeli, Turkey; 2) General Surgery Department, Faculty of Medicine, University of Kocaeli, Kocaeli, Turkey; 3) General Surgery Department, Faculty of Medicine, University of Istanbul, Istanbul, Turkey.

The C677T and A1298C mutations in the methylenetetrahydrofolate reductase (MTHFR) gene are involved in upto 65%; reduced enzyme specific activity, hyperhomocysteinemia, increased risk for venous thrombosis and atherosclerosis in homozygotes as well as in the compound heterozygotes. The C677T mutation in homozygotes is associated with bilateral breast cancer or combined breast and ovarian cancer. We investigated the frequency of C677T and A1298C mutations and their association with disease pattern in 87 Turkish breast cancer women and 103 controls. Population frequencies for the MTHFR C677C, C677T, and T677T genotypes were 50.6%, 33.3%, and 16.1% in the cases and 53.4%, 40.8%, 5.8% in the controls, respectively. The allelic frequency of C677T was 32.76% in the cases and 26.21% in the controls. Population frequencies of MTHFR A1298A, A1298C, and C1298C genotypes were 42.5%, 39.1%, and 18.4% in the cases and 48.5%, 45.6%, and 5.8% in the controls. The allelic frequency of A1298C was 37.93% in the cases and 34.47% in the controls. The MTHFR T677T/A1298A genotype was higher among 87 breast cancer patients compared with 103 controls, conferring a 26.90-fold increase in risk for breast cancer (OR = 9.685; 95% CI = 2.135-43.924, $X^2 = 12.245$, $P = 0.000$). We also observed a 10.44-fold increase in risk for breast cancer in individuals with the MTHFR C677C/C1298C variant allele (OR = 3.759; 95% CI = 1.296-10.905, $X^2 = 6.618$, $P = 0.010$). Individuals with the MTHFR C677C/A1298A genotype have a 2.36-fold protection against breast cancer ($X^2 = 6.226$, $P = 0.013$). In conclusion, folic acid metabolism appears to be important in breast cancer. Both individuals with the MTHFR T677T/A1298A, and C677C/C1298C genotypes provide susceptibility to breast cancer. However, individuals with the MTHFR C677C/A1298A genotype have protection against breast cancer.

Minimal expression of von Hippel-Lindau disease. *M.L. McMaster¹, G.M. Griffith², P.L. Choyke³, G.M. Glenn¹, W.M. Linehan².* 1) Genetic Epidemiology Branch, DCEG/NCI/NIH, Bethesda, MD; 2) Urologic Oncology Branch, CCR/NCI/NIH, Bethesda, MD; 3) Diagnostic Radiology Department, Warren Grant Magnuson Clinical Center, NIH, Bethesda, MD.

Introduction: In a family with a history of von Hippel-Lindau disease (VHL), an affected first degree relative clearly indicates the need for genetic and clinical screening studies, including CT and MRI. If the manifestations of VHL are mild, or even absent, there may be no suspicion of the risk of VHL in either the index or other generations. We report a series of patients with molecular documentation of VHL disease who have clinically silent or "minimal expression of VHL" -- a presentation that may delay diagnosis in the patient and forestall screening of their at-risk family members. **Methods:** Between 1989 and 2000, 756 patients from 215 kindreds with VHL underwent screening. Affected patients over 26 years were categorized as minimally, moderately or severely affected with VHL disease. A patient with minimal disease was defined as one being more than 26 years old, having a confirmed genetic diagnosis of VHL, and absent or mild clinical findings of VHL disease (limited to asymptomatic small lesions in two or fewer target organs). **Results:** Eight patients fulfilled the criteria for minimal expression of VHL disease. These patients ranged in age from 30 to 70 years, and five of the eight were female. Three of these patients had descendants with moderate or severe manifestations of VHL and five had no offspring. **Conclusion:** Minimal expression of VHL underscores the need for genetic testing of relatives of individuals with VHL disease, including asymptomatic family members who may be silent carriers.

Estradiol and tamoxifen stimulate lymphangiomyomatosis-associated angiomyolipoma cell growth. *J.J. Yu, A.G. Aristotelis, S. Howard, E.P. Henske.* Human Genetic Program, P-3045, Fox Chase Cancer Ctr, Philadelphia, PA.

Lymphangiomyomatosis is a progressive and often-fatal lung disease characterized by diffuse pulmonary smooth muscle cell proliferation and cystic degeneration of the lung parenchyma. Lymphangiomyomatosis occurs in women with tuberous sclerosis complex, and also in women without tuberous sclerosis complex (sporadic lymphangiomyomatosis). Renal angiomyolipomas occur in 50%-60% of sporadic lymphangiomyomatosis patients. We report here the establishment of a primary cell culture from a sporadic lymphangiomyomatosis associated renal angiomyolipoma. The angiomyolipoma cells contain somatic mutations inactivating both alleles of TSC2: a missense change in one allele (Arg611Gln), and loss of heterozygosity of the remaining allele. The cells expressed estrogen receptor alpha, estrogen receptor beta, and androgen receptor. The growth of these cells was stimulated by estrogen, associated with an increase in c-myc expression, suggesting that estrogen stimulates the growth of angiomyolipoma cells through transcriptional activation of estrogen responsive genes. Tamoxifen citrate also stimulated growth of the cells, indicating that tamoxifen has agonist effects on angiomyolipoma cells. These data provide the first in vitro evidence that estrogen stimulates the growth of angiomyolipoma cells. The smooth muscle cells of angiomyolipomas and pulmonary lymphangiomyomatosis appear to be closely related. Therefore, estrogen responsiveness could be related to the many reports of worsening of angiomyolipoma and lymphangiomyomatosis symptoms during pregnancy, and to the underlying reasons that lymphangiomyomatosis affects primarily women.

A Genome-Wide Analysis of Allelic Loss In Rhabdoid Tumor. *T.T-L. Chen¹, P. Grundy², J.A. Biegel³, G.E. Tomlinson^{1,4} and Hamon center, UT Southwestern Medical Center.* 1) Hamon Center , UT Southwestern Medical Center, Dallas, TX; 2) Cross Cancer Institute, Edmonton, AB; 3) Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania; 4) Department of Pediatrics, UT southwestern Medical Center, Dallas, TX.

Rhabdoid tumor of the kidney is a rare but highly aggressive malignant neoplasm of kidney. The development of rhabdoid tumor is associated with mutation of hSNFs/INI1 gene, which may be germline or acquired. In order to determine the extent and spectrum of other genetic change in rhabdoid tumors, we performed high-resolution genome-wide allelotyping for sixteen primary rhabdoid tumors and their matched genomic DNAs. DNAs was analyzed using 399 fluorescent microsatellite markers from the ABI Prism linkage mapping set v.2 on an ABI 377 sequencer/genotyper. Loss of heterozygosity(LOH) was observed in 31% of tumors in 22q11-12 region. Seven other regions with more than 10% of LOH were identified. The chromosomal arms with LOH were 1q42(12.5%), 2q24(12.5%), 4p16(18.75%), 4q12(12.5%), 11q14(12.5%), 15q26(12.5%), 16p12(12.5%), 22q11-12(31.25%). In one of primary tumor, there was discontinuous allele loss at several sites. We conclude that: Chromosome 22q11-12 allele loss is the primary recurring event in rhabdoid tumor corresponding to the loss of hSNF5/INI1 gene. Other losses were seen at chromosome 1q,2q,4p,4q,11q,15q,16p that might have a lesser role to play in rhabdoid tumor development.

Pooled analysis of loss of heterozygosity in breast cancer provides comparative evidence for multiple tumor suppressors in a genomewide scan and identifies novel candidate regions. *F.A. Wright¹, B.J. Miller², D. Wang¹, R. Krahe³.* 1) Dept of Biostatistics, Univ North Carolina, Chapel Hill; 2) College of Medicine, The Ohio State University, Columbus; 3) Section of Cancer Genetics, Dept of Molecular Genetics, University of Texas M. D. Anderson Cancer Center, Houston.

Somatic loss of heterozygosity (LOH) has been widely reported in breast cancer as a means of identifying putative tumor suppressor genes. However, individual studies have rarely spanned more than a single chromosome, and the varying criteria used to declare LOH complicate efforts to formally differentiate regions of consistent vs. sporadic (random) loss. Here, we report the first compilation of an extensive database from 151 published LOH studies in breast cancer, with summaries of over 15,000 tumors and 4,300 primary allelotypes. Allelic loss was evaluated at 1,173 marker loci, with large variation in marker density across the genome. An initial genome-wide analysis of the summarized loss data was performed, accounting for heterogeneity across studies and seeking direct evidence for preferential loss at each locus compared to nearby loci. A full likelihood-based approach using a chromosomal breakpoint and selection model was also performed, using studies in which primary allelotype information was available. The approach enables the direct comparison of candidate regions across the genome. Striking and significant evidence of preferential loss was observed on chromosomes 7q, 16q, 13q, 17p, 8p, 21q, 3p, 18q, and other regions, in many cases coinciding with previously identified candidate genes or known fragile sites. We report strong evidence for several tumor suppressor genes, but suggest that some previously-reported LOH results may not be systematic or reproducible.

PTEN is Targeted for Loss During Melanoma Tumor Development. *J. Stahl¹, M. Cheung¹, A. Sharma¹, S. Shanmugam¹, G.P. Robertson^{1,2,3}.* 1) Department of Pharmacology; 2) Pathology and Dermatology; 3) The Foreman Foundation for Melanoma Research, The Pennsylvania State College of Medicine, Hershey, PA 17033.

Malignant melanoma is the seventh most common cancer in the United States, affecting 1 in 71 Americans. Statistically one American dies from melanoma every hour; with an estimated 47,700 new cases and 7,700 deaths anticipated this year. Currently, there is no effective long-term treatment for patients suffering from the metastatic stages of this disease. Deletion or alteration of chromosome 10 occurs in 30-60% of sporadic human melanomas. The PTEN gene, located at 10q23, has been suggested to be a key factor on chromosome 10 involved in melanoma development. However, no biologically relevant model demonstrating suppression of melanoma tumor formation by PTEN or dissection of the mechanism underlying this process in melanomas has been reported. To investigate the functional involvement of PTEN in melanoma tumorigenesis, we have created an in vivo model in which a wild-type chromosome 10 was transferred into melanoma cells, and tumor development measured. We observed that tumor formation would not occur unless the cells first eliminated PTEN in order to relieve the growth suppressive effects of this gene. This has led to the establishment of a relevant model for studying PTEN involvement in melanoma since the chromosomally expressed gene is not overexpressed; rather it is produced at normal physiological levels even though only a single copy of the gene is present. Furthermore, it undergoes appropriate processing, and is under normal regulatory control. This approach has also been useful for creating isogenic cell lines to dissect the biological consequences of PTEN expression on tumor development as well as for determining the mechanism by which PTEN inhibit melanoma development. Different genetic targeting mechanisms such as deletion and epigenetic silencing were found to eliminate functional PTEN protein prior to tumor formation. Since these genetic events mirror those occurring in clinical tumor samples for targeting the PTEN gene, we believe that this is a relevant model for studying PTEN signaling and functioning in melanoma tumorigenesis.

DNA integrity as a potential apoptotic marker for the detection of colorectal cancer by analyzing stool DNA. *K.A. Boynton¹, I. Summerhayes², D.A. Ahlquist³, A.P. Shuber¹*. 1) Applied Research, EXACT Sciences, Maynard, MA; 2) Department of Urology, Lahey Clinic, Burlington, Massachusetts, 01805; 3) Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, Minnesota 55905.

Dysregulation of apoptosis is an important factor in the progression of colorectal cancer (CRC). As there may be less degradation of tumor DNA in CRC cells due to abrogated apoptotic dynamics, we have developed a methodology to analyze the integrity of DNA fragments purified from stools of CRC positive and colonoscopically negative patients to determine if DNA integrity is associated with the presence of disease. We analyzed the integrity of DNA purified from the stools of 27 CRC patients and 77 colonoscopically negative patients using this method. Stool DNA was purified by performing oligonucleotide-based hybrid DNA captures and long PCR was used to analyze the integrity of genomic DNA at four unique human genomic loci. Analysis of PCR amplicons indicates that DNA isolated from patients with CRC contains high molecular weight fragments, while DNA from colonoscopically normal patients contains significantly less or lacks high molecular weight DNA fragments. High molecular weight DNA fragments were found in 15 CRC stools (15/27, 56% sensitivity) and two colonoscopically negative stools (2/77, 97% specificity). These data suggest that fecal DNA integrity can be used to discriminate between individuals with cancer and those that are disease free. DNA integrity was also analyzed in a model system consisting of culture cells treated with Genistein, an apoptosis inducer. DNA fragment length was found to decrease when culture cells were exposed to Genistein. Integrity of DNA purified from stool as a novel marker may provide a sensitive method for the detection of apoptotic changes in the colon providing a means for the detection of CRC.

Identification of novel genes with somatic frameshift mutations within coding mononucleotide repeats in colorectal tumors with high microsatellite instability. *M. Ravnik-Glavac*^{1,2}, *U. Potocnik*^{1,3}, *D. Glavac*¹. 1) Department of Molecular Genetics, Institute of Pathology, Medical Faculty, Korytkova 2, Ljubljana, Slovenia; 2) Institute of Biochemistry, Medical Faculty, Ljubljana, Slovenia; 3) Laboratory of Genomic Diversity, NCI-FCRDC, Frederick, MD.

We have systematically retrieved genes with coding mononucleotide repeats from sequence databases and analysed them for mutations in MSI-H tumors. We found somatic frameshift mutations in 7/13 (77%) genes previously not analysed in MSI-H tumors. According to frequency of mutations in MSI-H tumors these genes could be divided into genes with high coding mononucleotide repeat instability (CMRI-H) and genes with low coding mononucleotide instability (CMRI-L). CMR-H genes were mutated in more than 24%; and CMRI-L in less than 11(%); of MSI-H tumors. Four genes in our study were CMRI-H and were therefore suggested to play a role in development of MSI-H tumors: TFE3(24%), TEF4(32%), RGS12(29%) and TCF132%). Our study suggests that systematic identification of genes with CMR in the sequence databases and determination of mutation frequency in MSI-H tumors might be powerful tool for identification of new molecular targets in development of MSI-H tumors.

Role of SV40 insertion site in the immortalization of human fibroblasts: Cloning and analysis of disrupted loci.

R.S. Athwal, J. Liu. Fels Inst Cancer Research, Temple Univ Sch Medicine, Philadelphia, PA.

Normal human diploid fibroblasts (HDF) multiply for finite generations and then enter senescence. Spontaneous transformation and immortalization have never been observed in HDF. Cells transformed with oncogenic DNA viruses such as SV40, Adv, and HPV can multiply for additional 20-30 doublings and give rise to immortal clones, albeit at a very low frequency. These studies suggest that alterations in cellular genes are essential for a cell to gain indefinite proliferation. Viral proteins are known to sequester p53 and Rb proteins and abrogate growth regulatory pathways controlled by these genes. We hypothesize that site of viral integration is another event that plays role in the regulation of normal cell growth. Following the identification of cell lines carrying a single copy of the ori SV40 viral genome, we applied inverse PCR to rescue human genomic sequences flanking the insertion site. PCR primers designed from the nucleotide sequence of the 500bp rescued fragment were used to screen a BAC library. The results of these studies identified a 150Kb BAC clone located at 1q21. The sequence of this region is available in the human genome database. We have developed a high-resolution physical, genetic and expression map of the region surrounding the BAC clone. This genomic region contains four cDNAs with open reading frames, which translate into hypothetical proteins. Experiments are underway to test BAC and full length cDNA clones for function in cell lines lacking the expression of these cDNAs. Rearrangements at 1q21 in breast tumors and abnormal expression of genes located in this region in transformed cells suggest a role for this region in the immortal growth of cells.

Identification of A Cell Senescence Gene at 6q16.3 for Ovarian Cancer. *V.K. Zhawar¹, N. Rane¹, A. Sandhu², G.P. Kaur¹, R. Athwal¹.* 1) Fels Inst Cancer Research, Temple Univ, Philadelphia, PA; 2) Wistar Institute, University of Pennsylvania, Philadelphia, PA.

Applying a Functional Positional approach, we have identified a genomic clone that carries a cell senescence gene, SEN6A, for ovarian tumor cells. The introduction of chromosome 6 or 6q into human (SKOV.3) and rat (ROSE199 and ROV12) ovarian tumor cell lines led to the restoration of normal cell growth phenotypes and senescence. Analysis of spontaneous revertant clones, which occurred due to deletions in the donor chromosome, allowed us to map the location of SEN6A locus within a 1cM genetic interval at 6q16.3. Precise localization of the deletions allowed us to identify three overlapping Yeast Artificial Chromosome (YAC) clones, corresponding to the smallest deletion, shared among independent revertant clones. Functional testing of candidate YAC clones identified a YAC that restored normal cell growth and senescence in ovarian tumor cells. Following the library screen, we assembled a contig of 17 BAC clones corresponding to the complementing YAC. Candidate BAC clones were tested for function by introduction into ovarian tumor cells. In these experiments two BAC clones were identified to carry SEN6A gene. The sequence of this 1cM region is now available in the human genome database. 25 ESTs, 11 unigenes and one full length cDNA, located in the candidate region have been assembled into a high resolution expression map. In an effort to identify candidate cDNAs, we have performed Southern blot analysis of tumor cell line, for a 1Mb region, using cDNA probes. These data revealed chromosomal rearrangements in a breast cancer cell line, MCF.7 and a hepatoma cell line, FOCUS for one of the cDNAs. Northern blot analysis of a multi-tissue blot with same cDNA identified transcripts of 6Kb and 4.5Kb size, expressed in skeletal muscle, pancreas and placental tissue. Our effort is directed to analyze the entire 1Mb region in tumor tissues for possible deletions or alteration in expression. A full-length candidate cDNA has been cloned into a mammalian cell expression vector for functional analysis.

Detection of *RB1* Mutations in Retinoblastoma patients. *E. Braggio*^{1,2}, *C.R. Bonvicino*^{1,3}, *H.N. Seúanez*^{1,2}. 1) Genetics Department, Instituto Nacional de Câncer, Rio de Janeiro, Brazil; 2) Genetics Department, Universidade Federal do Rio de Janeiro, Brazil; 3) Tropical Medicine Department, IOC-Fundação Instituto Oswaldo Cruz, Rio de Janeiro, Brazil.

Retinoblastoma is the most common intraocular tumor in children arising almost exclusively under 5 years of age. Both copies of *RB1*, the gene responsible for this tumor, must be functionless for the development of this malignancy. To detect mutations in the *RB1* gene in 27 patients (15 with bilateral and 12 with unilateral retinoblastoma) we isolated DNA from formalin-fixed, paraffin-embedded tissue and screened the entire coding region (exons) and nearby intronic regions by PCR-mediated SSCP analysis followed by direct DNA sequencing. We identified 2 novel mutations and 8 previously described in 10 patients, all of which consisting of single base substitutions. One of the novel mutations involved a G→A transition in the last, invariant base of intron 13 (G76429A) affecting mRNA splicing. The other, a T→C transition in exon 20, was a T156795C resulting in L679P. Both were described in unilaterally affected patients and we confirmed the germ-line origin of the first mutation in DNA extracted from a blood sample. The eight others were C→T transitions that changed CGA-arginine codons to TGA-stop codons and were localized in exon 10 (C64348T; R320X), 14 (C76430T; R445X), 17 (3 mutations C78238T; R552X and C78250T; R556X), and 18 (2 mutations C150037T; R579X). Although specific mutation hot spots have not been identified in the literature 8 of 10 mutations occurred in CGA codons and 7 in E1A binding domains, with 5 mutations affecting CGA codons within E1A binding domains (codons 393-572 and 646-772). These sites are apparently more frequent mutational targets and should be initially screened in patients with retinoblastoma.

Is age of onset in breast cancer families heritable? *Y. Yao*^{1,2}, *D. Markakis*¹, *B. Doan*¹, *K. Helzlsouer*¹. 1) Epidemiology, Johns Hopkins Univ, Baltimore, MD; 2) Center for Inherited Disease Research, Johns Hopkins Univ, Baltimore, MD.

The goal of this study is to test whether age of onset in breast families is heritable. We recently performed a correlation study of age of onset involving 629 breast cancer families from the Johns Hopkins breast and ovarian surveillance service (BOSS). The analyses were performed with the FCOR2 program of SIBPAL2 only including relative pairs with documented age of onset and verified cancer status. The correlation coefficients (CC) for different types of relatives are 98.9%, 38.1%, 23.5%, 66.7% and 39.3% for father:son, father:daughter, mother:daughter, sister:brother and sister:sister pairs. Interestingly, grandmother:granddaughter pairs correlated differently in the pairs connected through father and the ones connected through mother (-29.6% vs 11.6%). CC for paternal aunt:niece and maternal aunt:niece were 20.3% and 21.6%, respectively. These results support the hypothesis that age of onset is heritable. The confirmation of this finding using different high-risk breast cancer registries is necessary and updated results will provide further motivation to look for genetic modifiers that interact with BRCA1 and BRCA2 mutations and help distinguish families with various level breast cancer risk in genetic counseling.

Detecting genetic modifier involved in extracolonic manifestations in Familial Adenomatous Polyposis (FAP) families: Power to detect linkage in 22 eligible families. *B. Doan*^{1,2}, *F. Giardiello*³, *Y. Shugart*^{1,2}. 1) Department of Epidemiology, Bloomberg School of Public Health Johns Hopkins Univ, Baltimore, MD; 2) Dept ICM/CIDR, Johns Hopkins Univ, Baltimore, MD; 3) Department of Medicine, Johns Hopkins Univ, Baltimore, MD.

FAP is caused by germline mutations in the adenomatous polyposis coli (APC) gene on 5q21. Although the hallmark of FAP includes innumerable adenomatous polyps, the disease phenotype often includes the presence of one or more extracolonic symptoms. With genetic testing available for identified at risk patients to undergo rigorous screening and prophylactic colectomy, the presence of some extracolonic symptoms have become the leading cause of further morbidity and mortality. This study is designed to search for new genes associated with these extracolonic manifestations based on the assumptions that these genes act in concert with the APC mutated gene to contribute to the phenotype variability. This first step is to identify eligible families from the Johns Hopkins University Hereditary Colorectal Cancer Registry. Patients were originally recruited from a 5 state area (MD, VA, DE, PA, WV) and D.C. The Registry includes: 1) family histories and medical records on patients and 2) families with a history of familial aggregation of colorectal cancer. We have identified 22 FAP families with at least 3 affected siblings, each carrying APC mutations and having at least one extracolonic manifestation. To determine the strategy for gene mapping, we performed simulations, followed by lod score analyses, considering heterogeneity. Results showed that the power to detect linkage is 72% with an ELOD of 4.2 if 80% families are linked; the power is 45% with an ELOD of 3.02 if 70% pedigrees are linked and the power reduces to 25% with an ELOD of 2.10 if only 50% pedigrees are linked. Our results indicate that we have collected a set of eligible families with reasonable power for a stage I genome scan although more families are being identified to improve power. Further, we will try to collect important information such as age of onset and smoking status, which will lead to parametric and non-parametric linkage analysis with covariates.

SNP500Cancer database and candidate SNP approach to genetic association studies. *M. Yeager*^{1, 2}, *B. Packer*², *E. Miller*², *R. Welch*², *M. Kiley*², *M. Burke*², *R. Strausberg*³, *N. Rothman*⁴, *S. Chanock*¹. 1) Section on Genomic Variation, Pediatric Oncology Branch, NCI, Bethesda, MD; 2) Intramural Research Support program, SAIC Frederick, NCI-FCRDC, Frederick, MD; 3) Office of Cancer Genomics, NCI, Bethesda, MD; 4) DCEG, NCI, Bethesda, MD.

SNP500Cancer, an integral part of the long-term Cancer Genome Anatomy Project, is a new database designed to publicly address the immediate needs for candidate SNP and candidate gene approaches to mapping complex diseases. SNP500Cancer provides bi-directional sequencing information on a set of control DNA samples derived from anonymized subjects (102 Coriell samples representing 4 self-described ethnic groups: African/African-American, Caucasian, Hispanic and Pacific Rim). All SNPs are chosen from public databases and reports, and the choice of genes includes a bias towards nonsynonymous and promoter SNPs in genes that have been implicated in one or more cancers. The website (<http://snp500cancer.nci.nih.gov>) is searchable for SNPs by gene, chromosome and by known dbSNP ID. The database currently contains over 1100 SNPs, and as of June 1, 2002, 531 SNPs have been sequenced. For each analyzed SNP, gene location and over 200 bps of surrounding annotated sequence (including nearby SNPs) are provided, with frequency information in total and per sub-population. A calculation of Hardy-Weinberg Equilibrium is included. Moreover, genotyping assays (5-exonuclease, hybridization-triggered fluorescence, and/or MALDI-TOF) are developed for SNPs with minor allele frequencies > ~5%. These assays are performed on the same 102 samples and the results are compared for concordance with the sequencing results. The website will soon post the conditions (including sequences for probes, primers, etc.) for validated genotyping assays. Estimation of haplotypes is currently provided for select genes, with the goal of expanding this effort rapidly. SNP500Cancer provides an invaluable resource for investigators world-wide to select SNPs for analysis, design genotyping assays using validated sequence data, choose selected assays already validated on one or more platforms, and provide reference standards for genotyping assays of interest.

Second primary cancers in men with prostate cancer: An increased risk for male breast cancer - a key to identify BRCA2 families? *C. Thellenberg, B. Malmer, B. Tavelin, H. Grönberg.* Department of Radiation Sciences, Oncology, University of Umeå Sweden.

Background: Studying second primary cancers can give valuable insight in etiology, as genetic factors in common and shared risk factors for the first and the primary cancer. Second primary cancers following prostate cancer have been studied earlier and most studies have found decreased overall risks for second primary cancers, however increased risks have been found for urinary bladder cancer and kidney cancer. The aim in this study is to examine the risk for second primary cancers following prostate cancer, in a large population based cohort in order to find clues to etiological factors in common. **Method** All prostate cancer cases in the Swedish Cancer Registry from 1958 to the end of 1996 constituted the study base, (n = 135.713) were included. The risk (Standardized Incidence Ratio, SIR) of second primary cancers was calculated as the ratio between observed and expected number of cancers. Two-tailed 95% confidence intervals (CI) were used to test the significance. **Results:** The most interesting finding was an increased risk (SIR 2.01 95% CI 1.44-2.74) for developing male breast cancer. The risk was more pronounced in younger ages indicating at least partly a genetic explanation to the increased risk. An overall increased risk (SIR 1.17, 95 % CI 1.15-1.19) for second primary cancers were found, but the risk was only seen in the first six months of follow-up (SIR 3.45, 95% CI 3.32-3.57). **Conclusions:** There is an increased risk for second primary male breast cancer following prostate cancer. This association might be due to BRCA2 mutation and have not been reported earlier. A more detailed investigation is essential to confirm this finding.

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Candidate Gene Linkage Analysis in a Prostate Cancer Affected Sib Pair Population. *G.P. Larson, T.G. Krontiris.*
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Prostate cancer is the second leading cause of cancer mortality in American males. Epidemiological studies of twins suggest a strong inherited genetic component to disease susceptibility; however, genome-wide linkage analysis approaches have found few loci implicated in disease. Recently low-penetrance risk variants in this disease (RNASEL, HPC2) and diseases such as inherited ovarian fibroid disease have been identified. In these cases, mutations in genes involved in routine aspects of metabolism were identified and suggest that a candidate disease approach utilizing genes integral to such processes as DNA integrity, steroid metabolism, and cell-cycle control will be successful in identifying low-penetrance disease alleles. We present results that support linkage for multiple candidate genes and their regions including 10q21 and 3p14 by utilizing a physically dense spectrum of markers throughout the candidate interval. The maximum NPL identified utilizing GENEHUNTER is approximately 2.3 for the 10q21 region. This result is further supported by single-point IBD analysis in SIBPAL over a physically distinct, subcentimorgan interval. We are refining the region utilizing a dense set of SNP markers to identify areas of linkage disequilibrium in this cohort with the ultimate goal of identifying disease variants in our candidate gene(s).

Linkage analysis of prostate cancer susceptibility: Confirmation of linkage at 8p22-23. *F. Wiklund, B.A. Jonsson, I. Goransson, H. Gronberg.* Oncology, Radiation Science Univ Umea, Umea, Sweden.

BACKGROUND: Evidence for a prostate cancer susceptibility locus at the short arm of chromosome 8 was reported in a recent study. They performed linkage analysis, using 21 densely spaced markers spanning ~35 cM at 8p22-23, in 159 pedigrees affected by hereditary prostate cancer (HPC). In the present report we replicate the linkage analysis in 61 Swedish pedigrees with HPC.

AIM: To confirm linkage to 8p22-23 in 61 Swedish pedigrees with HPC.

METHODS: Thirteen microsatellite markers spanning ~35 cM at 8p22-23 were genotyped in 61 Swedish pedigrees with HPC. Both parametric and nonparametric multipoint linkage analyses were performed, implemented by the computer program GENEHUNTER-PLUS.

RESULTS: Both parametric and nonparametric analyses confirmed evidence for linkage between a prostate cancer susceptibility locus and markers on chromosome 8p in the complete 61 HPC pedigrees. The highest parametric HLOD was 0.58 ($P = 0.050$) with $a = 0.22$, observed at D8S1731. The nonparametric analyses yielded a peak allele-sharing LOD of 0.67 ($P=0.038$) at marker D8S1731. When the analyses were stratified by pedigree characteristics, the evidence of linkage was seen in pedigrees with early onset and in pedigrees with small number of affected. The peak allele-sharing LOD was 1.07 ($P=0.013$) at D8S552 in the 16 pedigrees with mean age at onset < 65 years, and 1.39 ($P=0.006$) at D8S1731 in the 47 pedigrees with less than five affected pedigree members.

CONCLUSIONS: Our study confirms previously reported evidence of prostate cancer linkage at 8p22-23 in an independent population. These results, together with the identification of frequent somatic alterations of 8p loci in prostate cancer cells and the discovery of mutations in some tumor-suppressor genes in the region, warrant further studies.

A conserved domain within Snm1 is required for the repair of DNA interstrand cross-links. X. Li¹, L. Thrun¹, D. Bruun¹, S. Jones², R. Moses¹. 1) Dept. of Molecular & Medical Genetics, Oregon Health & Science University, Portland, OR; 2) Dept. of Cell Biology, University of Massachusetts Medical Center, Worcester, MA.

DNA interstrand cross-links (ICLs) block transcription, replication and segregation of DNA. *SNM1* of yeast is required for normal ICL repair and homologs exist from yeast to human. The function of Snm1 is unknown. *SNM1* shows some homology to *Artemis*, acting in V(D)J recombination. Mutations in *Artemis* cause human radiosensitive severe combined immune deficiency (RS-SCID). In response to treatment with psoralen plus UVA radiation, *snm1* mutant yeast cells were similar to wild type cells in inducing chromosomal breaks as analyzed by pulsed-field gel electrophoresis (PFGE), but they were defective in the subsequent repair of such breaks, suggesting Snm1 is required for processing of ICL-induced chromosomal breaks. Double-stranded breaks (DSB) are thought to be an intermediate in repair of ICLs, but we found Snm1 is not required for the repair of mating type switch endonuclease HO-induced DSBs. These results suggest that ICL-induced DNA breaks (as repair intermediates) are structurally different from DSBs induced by HO, or that SNM1 is not involved in the DSB repair step. A recent report (Ma et al. Cell 108: 781-94) showed that both Snm1 and Artemis contain b-lactamase motifs and that Artemis possesses a single-strand exonuclease activity, possibly acting in processing intermediates in recombination. We find that a point mutation in motif-2 of the b-lactamase domain abrogates functional complementation for ICL repair in a *snm1* deletion mutant, indicating this motif is important for the action of Snm1. We have made SNM1^{-/-} mice in which the gene was disrupted upstream of all of the lactamase motifs. Results show *snm1*^{-/-} fibroblast cells are sensitive to the ICL agent CDDP. However, FACS studies with T-cell and B-cell-specific markers indicate no abnormality. Our results do not support a role for *SNM1* in immunocompetence, but do indicate the b-lactamase domain is required for function in ICL repair.

Heterogenous Expression of beta-Catenin, p16, E-cadherin, and C-myc in Multistage Colorectal Carcinogenesis Detected by Tissue Microarray. *D. Xie¹, W.F. Zeng², J.S.T. Sham¹, H.L. Lin², L.H. Che², Y. Fang³, J.M. Wen², L. Hu¹, X.Y. Guan¹.* 1) Dept. of Clinical Oncology, The University of Hong Kong, Hong Kong, China; 2) Department of Pathology, Sun Yat-Sen University, Guangzhou, China; 3) Cancer Institute, Sun Yat-Sen University, Guangzhou, China.

Most colorectal carcinomas (CRCs) arise from adenomas through an archetypal pathogenic pathway, the adenoma-carcinoma-metastasis sequence. Aberrant expressions of b-catenin, p16, E-cadherin, and c-myc have played important roles in carcinogenesis of CRC, but their distribution pattern and associations in different pathological loci along CRCs tumorigenetic progress is not fully understood. In this study, a tissue microarray (TMA) containing 85 advanced CRCs in different Dukes stages was constructed. In all 85 cases, tissue specimens from normal mucosa, primary carcinomas in different layers of the bowel wall were included in the TMA. Tissue specimens from matched adenoma, lymph node metastases, and distant metastases were recruited from 22, 21, and 21 cases, respectively. Expression pattern of b-catenin, p16, E-cadherin, and c-myc in multistage colorectal carcinogenesis and progression was evaluated by immunohistochemistry. The results revealed 1) Nuclear overexpressions of b-catenin, p16, and c-myc was increased apparently from normal mucosa to adenoma, primary carcinoma, and lymph node metastatic tumor; 2) The frequencies of nuclear overexpression of b-catenin and p16 in lymph node metastases were significantly higher than that in distant metastases ($p < 0.05$). This result implies that nuclear overexpression of b-catenin and/or p16 might be associated with CRCs local lymph node metastasis but probably not distant blood-bore metastasis; 3) A well correlation of nuclear expressions of b-catenin and c-myc either in primary carcinomas at subserosa layer or lymph node metastases was observed ($p < 0.05$), suggesting overexpression of c-myc could be transcriptionally activated by nuclear b-catenin in both primary CRC and its nodal metastases.

Comparative Expression Profiling of the Mouse Cytochrome P450 Gene Family. *I. Stoilov¹, D. Choudhary², I. Jansson², J.B. Schenkman², M. Sarfarazi¹.* 1) Molecular Ophthalmic Genetics Laboratory, Surgical Research Center, University of Connecticut Health Center, Farmington, CT; 2) Department of Pharmacology, University of Connecticut Health Center, Farmington, CT.

Expression profiles for 48 mouse genes representing all known cytochrome P450 families were developed with the assistance of multiple tissue cDNA panel Mouse I (Clontech). This panel contains normalized cDNA samples from 4 embryonic stages (days 7, 11, 15 and 17) and 8 adult tissues: heart, brain, spleen, lung, liver, skeletal muscle, kidney and testis. Individual gene-specific PCR assays were uniformed with respect to their primer T_m, amplicon size, reaction conditions and number of cycles used. The amplification reactions were analyzed by agarose/EtBr gel electrophoresis. Low-mass DNA ladder (Invitrogen) was used for band sizing and as a quantitative reference. Gel images were digitized and quantitative densitometry analysis was performed with Scion Image software program. The level of expression of each gene was translated into ng of RT-PCR product. Mouse P450s were detected in the gastrulation phase and number of expressed genes steadily increased during somitogenesis and organogenesis phase of development. In adult tissues, the largest number of P450s was detected in liver (n=34) followed by kidney (n=33), lung (n=27) and testis (n=26). Heart had the fewest number (n=12) of P450s. Of special interest was the expression profile of P450 genes belonging to sub-family Cyp1 because the mutant forms of Cyp1b1 ortholog in human cause Primary Congenital Glaucoma. Both Cyp1a1 and Cyp1b1 showed a complex non-overlapping pattern of expression during embryonic development. Cyp1a1 was detected only at embryonic day 7 while Cyp1b1 was detected on days 11, 15 and 17. In adult tissues, the highest Cyp1a1 expression was in lung and liver. Interestingly, Cyp1b1 was not detected in liver but was present in all other tissues. On the contrary, Cyp1a2 was detected only in the adult liver. This data suggests that Cyp1a1 and Cyp1a2 are not able to compensate for Cyp1b1 deficiency during embryonic development. Supported by AHAF-National Glaucoma Research grant to I.S. and NIH (EY-11095) grant to M.S.

Loss of heterozygosity in premalignant lesions and invasive tumors of the breast: examination of 26 commonly deleted chromosomal regions. *R.E. Ellsworth¹, C.D. Shriver², D.L. Ellsworth¹, B. Deyarmin¹, V. Mittal¹, S. Lubert¹, R.I. Somiari¹.* 1) Windber Research Institute, Windber, PA; 2) Walter Reed Army Medical Center Washington, D.C.

Despite tremendous efforts towards the elucidation of the genetic events involved in breast cancer etiology, the majority of molecular dysfunctions associated with breast cancer are still unknown. The successful sequencing of the Human Genome has revealed that tumor suppressor genes (TSGs) are often found clustered together in small chromosomal regions. We have taken a high-throughput genotyping approach to examine 26 chromosomal regions, including the BRCA1, BRCA2 and TP53 gene regions, frequently deleted in breast cancer tumors to identify TSGs and TSG gene clusters involved in a variety of breast cancers. Approximately 100 tumor samples have been studied, including cases of atypical hyperplasia, in situ carcinoma and infiltrating carcinoma (stages 0-3). DNA was extracted from relatively homogenous cell populations using laser microdissection technology. First-pass genotyping on a 96-capillary electrophoresis sequencer equipped with an ultra-high throughput genotyping software involved the use of two microsatellite primer pairs per chromosomal region for the development of a global LOH map. Fine mapping was then carried out on the subset of samples showing specific chromosomal loss using large, custom panels of microsatellites from each region. Of note, ~60% of cases of DCIS show a loss of heterozygosity on chromosome 8p22 with a commonly deleted region encompassing the deleted in liver cancer 1 (DLC1) gene. Development of this large map, representing 26 loci on 17 chromosomes, will greatly facilitate the identification of novel tumor suppressor genes and/or surrogate markers for breast cancer.

A common haplotype of CYP1B1 is associated with prostate cancer risk in Caucasians. *B. Chang¹, S.L. Zheng¹, S.D. Isaacs², K.E. Wiley², A.R. Turner¹, G.A. Hawkins¹, E.R. Bleecker¹, P.C. Walsh², D.A. Meyers¹, W.B. Isaacs², J. Xu¹.* 1) Center for Human Genomics, Wake Forest Univ Sch Med, Winston-Salem, NC; 2) Department of Urology, Johns Hopkins Medical Institutions, Baltimore, MD.

Cytochrome P450 1B1 has been hypothesized to play an important role in carcinogenesis because it catalyzes the conversion of 17- β -estradiol (E2) to the catechol estrogen metabolite 4-OH-E2, the most carcinogenic estrogen which induces DNA single-strand breaks. As recent epidemiological and animal studies suggest that estrogen metabolism is involved in prostate carcinogenesis, we hypothesize sequence variants of CYP1B1 affect estrogen metabolism and in turn affect prostate cancer risk. To test this hypothesis, we systematically genotyped 14 single nucleotide polymorphisms (SNPs) of CYP1B1 in 159 probands of hereditary prostate cancer (HPC) families, 245 sporadic prostate cancer cases, and 211 unaffected controls. These SNPs well cover the gene and include two SNPs in the promoter region (-1549G/A and -1001T/C), five nonsynonymous changes (R48G, A119S, V432L, D449E, and N453S), four in introns (IVS1-13T/C, IVS1-263A/G, IVS2+3015G/A, and IVS2+3653C/A), and three in the 3'UTR (+5359T/A, +5639G/A, +7072A/T). Because the majority of study subjects are Caucasians, all the hypothesis tests were limited to Caucasians. Significantly different allele frequencies between sporadic cases and unaffected controls were observed for five consecutive SNPs in the promoter region, intron 1, and exon 2 ($P < 0.05$). Due to strong linkage disequilibrium (LD) within these five SNPs, only two major haplotypes were observed (all other possible haplotypes were either not observed or observed in $< 1\%$). The haplotype of C-C-G-C-G of SNPs -1001T/C, IVS1-13T/C, IVS1-263A/G, R48G, and A119S were observed in 75% of sporadic cases, compared with 68% of controls ($P = 0.038$). These results suggest that sequence variants in CYP1B1 may be associated with prostate cancer risk.

Isolation of expressed sequences from a genomic segment carrying senescence gene (SEN16) for breast tumor cells. *G.P. Kaur, R. Athwal.* Fels Cancer Research Inst, Temple Univ Sch Medicine, Philadelphia, PA.

Escape from replicative senescence has been postulated to be a prerequisite for progressive tumor growth. Cells cultured from many tumors exhibit immortal cell growth in culture while all normal human cell types that can be grown in culture undergo cellular senescence after a definite life span. Cellular senescence is expressed as dominant phenotype over immortal cell growth, which indicates that immortal phenotype arises due to recessive genetic mutations in the senescence pathways(s). More than ten human chromosomes have been identified to carry senescence genes by complementation of immortal phenotype of a variety of cultured tumor cells. However, cloning of senescence genes has been a challenging task so far. We have applied an approach that combines functional complementation with traditional positional cloning to isolate human genes that induce senescence in immortal tumor cell lines. Using this functional-positional approach, we have identified a cell senescence gene, SEN16, located at chromosome 16q24 to a 85 kb genomic segment (Reddy et al.1999, Oncogene 18:5100, Reddy et al. 2000, Oncogene19: 217). A high-resolution map of the candidate region was constructed with YAC and BAC clones. Functional testing of these clones identified a genomic segment of 85 kb that could induce replicative senescence in human and rat mammary tumor cells. In following experiments, we isolated expressed sequences from the complementing BAC clone by exon trapping. Twelve exons were identified and their sequence comparison with databases identified five partial cDNA clones. Two of these cDNAs have been characterized in detail with respect to their transcript size and expression in different tissues. Both of these cover 55 kb of the complementing genomic region. One of these cDNA is rearranged in two breast tumor cell lines, SKBR3 and T47D, thus promises to be a more likely candidate. This cDNA has been cloned into LACII inducible expression system. Cloned cDNA will be expressed in tumor cells and resulting phenotype studied.

Novel germline mutations in the gene coding for α -Methylacyl-CoA Racemase may associated with prostate cancer risk. *S. Zheng¹, B.L. Chang¹, S.D. Isaacs², K.E. Wiley², A.R. Turner¹, G.A. Hawkins¹, E.R. Bleecker¹, P.C. Walsh², D.A. Meyers¹, W.B. Isaacs², J. Xu¹.* 1) Center for Human Genetics, Wake Forest Univ Sch Med, Winston-Salem, NC; 2) Department of Urology, Johns Hopkins Medical Institutions, Baltimore, MD.

The enzyme α -Methylacyl-CoA Racemase (AMACR) plays an important role in peroxisomal β -oxidation of branched-chain fatty acid and C27-bile acid intermediates, and therefore is relevant to carcinogenesis. The involvement of AMACR in prostate carcinogenesis is further implicated by the following several observations: 1) two recent studies independently found that AMACR is consistently over-expressed in prostate cancer at both the mRNA and protein level; 2) multiple epidemiological studies have found that red meat and dairy products, major sources of branched-chain fatty acid, are associated with prostate cancer risk; and 3) chromosomal region 5p13, the location of the AMACR gene, is linked to a prostate cancer susceptibility gene by two genome-wide scans. In this study, we hypothesize that sequence variants in AMACR may alter the risk for prostate cancer. To test for this hypothesis, we sequenced all 5 exons, exon-intron junctions, the promoter region, and both 5' and 3' UTR of AMACR in germline DNA samples of 96 probands from hereditary prostate cancer (HPC) families ascertained at the Johns Hopkins Hospital. We found 17 sequence variants, including 5 novel (R118Q, V185A, P238S, Q239H, L250R) and 5 known (V9M, S52P, G175D, S201L, K277E) nonsynonymous changes. Six of these are at the residues that are conservative among the rat and mouse Amacr. To assess whether these sequence variants are associated with prostate cancer risk, we genotyped a subset of these variants among an additional 63 HPC probands, 245 sporadic cases, and 211 unaffected controls. Several nonsynonymous changes were observed more frequently in cases than in controls. In particular, the nonsynonymous change S52P was only observed in two Caucasian cases (one each in HPC probands and sporadic cases), and never observed in 189 Caucasian unaffected men. These results suggest that sequence variants in AMACR may be associated with prostate cancer risk.

No association between germline sequence variants in Angiopoietin-2 gene and prostate cancer risk. *J. Xu¹, B.L. Chang¹, S. Zheng¹, S.D. Isaacs², K.E. Wiley², A.R. Turner¹, G.A. Hawkins¹, E.R. Bleecker¹, P.C. Walsh², D.A. Meyers¹, W.B. Isaacs².* 1) Center for Human Genomics, Wake Forest Univ Sch of Med, Winston Salem, NC; 2) Department of Urology, Johns Hopkins Medical Institutions, Baltimore, MD.

A gene or genes on chromosome 8p22-23 have been implicated in prostate carcinogenesis by the observation of frequent deletions of this region in prostate cancer cells, and by two recent linkage studies in hereditary prostate cancer (HPC) families. Angiopoietin-2 (Ang-2) is a good candidate gene in this region because of its role in angiogenesis, which is critical in tumor growth and metastasis. Multiple studies have shown that higher activity of Ang-2 may lead to enhanced tumor angiogenesis and growth. The Ang-2 gene consists of 9 exons and spans about 61 kb. So far, only two sequence variants have been reported in the coding sequence of Ang-2, and both are synonymous changes (Q245 and Y369). To identify additional sequence variants in prostate cancer patients, we sequenced all 9 exons, exon-intron junctions, the promoter region, and both 5' and 3' UTR of Ang-2 in germline DNA samples of 96 probands from hereditary prostate cancer (HPC) families ascertained at the Johns Hopkins Hospital. We found 26 sequence variants, including 4 variants in the 5'UTR, 4 variants in the 3'UTR, and 3 synonymous changes (T294, N365, and Y369). To assess whether these sequence variants are associated with prostate cancer risk, we genotyped a subset of these variants (n=12) among an additional 63 HPC probands, 245 sporadic cases, and 211 unaffected controls. There was no significant difference in the allele frequencies between prostate cancer cases and controls for any of these variants. These results suggest that sequence variants in Ang-2 are unlikely play a major role in affecting prostate cancer risk.

Genome wide screening for prostate cancer susceptibility loci: Results from the Mayo Clinic Familial Prostate Cancer Study. *J.M. Cunningham, R. Berry, J.J. Schroeder, A.J. French, A.F. Marks, S.J. Hebring, B.R. Koch, S.A. Anderson, S.K. McDonnell, B.J. Peterson, S.L. Slager, S.N. Thibodeau, D.J. Schaid.* Mayo Clinic, Rochester, MN.

Prostate cancer is one of the most common human cancers and has long been recognized to occur in familial clusters, with family members sharing an increased risk of cancer development. Genetic linkage is a powerful technique for identifying disease susceptibility loci, although it can be confounded by factors that are present in prostate cancer, such as late age of onset and a high phenocopy rate. Nonetheless, several putative loci have been identified on chromosomes 1 (HPC1, PCAP and CAPB), X (HPCX), 17 (HPC2) and 20 (HPC20), with genes for two, HPC1 and HPC2, being tentatively defined. We report herein on a genome-wide scan in 161 prostate cancer families, using the ABI Prism Linkage Mapping Set version 2 of 400 microsatellite markers. The most significant linkage was found for chromosome 20, with an Hlod (recessive model) of 4.70 ($\alpha = 0.30$), and an NPL of 3.58 for the whole group. Linkage was most prominent among families with a late age of onset (>66 years), with <5 affected family members and with no male to male transmission (MMT) of disease (Hlod = 3.94, $\alpha = 1.0$, NPL = 3.62). No other chromosome showed significant evidence for linkage within the entire group. However, chromosome 6 demonstrated some linkage potential, with an Hlod of 1.93 (recessive model) and an NPL of 1.8 for the whole group. Age appeared to influence this somewhat, with Hlod of 1.99 and an NPL of 2.8 in those with an older age of onset. Within the subsets described above, another region showing potential evidence for linkage was chromosome 5, in those families without the presence of MMT of disease (Hlod 3.28, dominant model, and an NPL of 3.61). Data for the entire genome wide scan will be presented along with appropriate subset information.

CYP3A and GST genotypes in treatment response in pediatric leukemia. *R. Aplenc*^{1, 2}, *K. Meredith*², *B.J. Lange*², *T. Rebbeck*¹. 1) CCEB, Univ Pennsylvania, Philadelphia, PA; 2) Children's Hospital of Philadelphia, Philadelphia, PA.

Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer. Present day therapies stratify patients according to clinical criteria and cure approximately 80% of patients. Despite risk stratification based on clinical criteria, some patients relapse. The risk of relapse in pediatric ALL may be related to polymorphisms in drug metabolizing enzymes. We evaluated polymorphisms in cytochrome P450 3A5 (CYP3A5) and glutathione-s-transferase (GST) and relapse and toxicity risk in pediatric ALL. CYP3A5 may be the primary Phase I enzyme involved in the metabolism of several chemotherapy agents to active Phase I metabolites. GST may be responsible for a majority of glutathione conjugation and deactivation of Phase II metabolites of chemotherapy agents. To date, no studies of CYP3A5 have been reported in pediatric ALL and controversy exists on the role of GST polymorphisms. We hypothesized that patients with deactivating SNPs in CYP3A5 would have less active chemotherapy metabolites and therefore a decreased risk of toxicity but an increased risk of relapse. Conversely, we hypothesized that patients with GST deactivating SNPs would have higher Phase I chemotherapy metabolite exposure with more attendant toxicity but a lower rate of relapse. The Children's Cancer Study Group (CCG) national ALL study, CCG-1891, enrolled 1204 standard risk patients from 1989 to 1992 of whom 222 patients relapsed. Archived bone marrow slides from patients enrolled on CCG-1891 were obtained. DNA was extracted by standard protocols. CYP3A5 genotype was determined by a Pyrosequencing assay. GST mu and theta genotypes were determined by a previously described assay. Approximately 100 relapse patients and 380 non-relapse patients were genotyped for each SNP. GSTM1, GSTT1, and CYP3A5*3 genotypes were not associated with statistically significant changes in relapse risk. (GST M1 OR = 1.25, 95% CI 0.76-2.08; GST T1 OR 0.59, 95% CI 0.24 - 1.29; CYP3A5*3 OR = 1.36, 95% CI 0.50 - 3.33). No interaction between CYP3A5 and GST genotypes was observed. We conclude that CYP3A5 and GST genotypes do not significantly modify the risk of relapse in children treated for standard risk ALL.

Expression profiling of normal breast fibroblasts from *BRCA1* mutation carriers following induced DNA damage: identification of genes predictive of the presence of mutation. Z. Kote-Jarai¹, N. Cattini², M. Copeland¹, I. Giddings², R. te Poele², R. Wooster², C. Campbell³, G. Gui⁴, J. Peacock⁵, R. Eeles^{1,4}. 1) Translational Cancer Genetics, Inst. Cancer Research, Sutton, Surrey, UK; 2) Molecular Carcinogenesis, Inst. Cancer Research, Sutton, Surrey, UK; 3) Computational Intelligence Unit, Bristol University, Bristol, UK; 4) Royal Marsden NHS Trust, London, UK; 5) Radiobiology Team, Institute of Cancer Research, Sutton, Surrey, UK.

Germline mutations in the *BRCA1* gene predispose women to develop breast and ovarian cancer. *BRCA1* has been implicated in many cellular processes including transcription regulation, DNA repair and recombination. We employed high-density cDNA microarray technology in order to identify different responses to DNA damage in heterozygous mutation carrier cells when compared to wild type cells. We used normal primary breast fibroblasts cultured from prophylactic mastectomy specimens of women carrying a germline mutation in the *BRCA1* gene. Control breast fibroblast cells were obtained from women undergoing reduction mammoplasties. Cells were subjected to 15 Gy of gamma-irradiation using a ⁶⁰Co source. Our cDNA arrays were produced from 5604 IMAGE clone cDNAs. The clone set includes specifically chosen genes such as the currently known *BRCA1* interacting genes and genes with known function in DNA repair. The PCR products were gridded onto poly-L-Lysine coated glass microscope slides. After hybridisation the slides were scanned using an Axon Genepix 4000A scanner and the images were analysed with GenePix Pro 3.0. Data mining was performed using GeneSpring 4.2 (Silicon Genetics). Based on the Class Prediction model we have identified a set of genes differentially expressed in *BRCA1* heterozygous mutation carriers when compared to non-carriers. These predictor genes represent transcription regulators, cell cycle control genes and genes with function in chromatin remodelling. We are currently extending our study to establish the significance of these predictor genes. This result shows that gene expression profiling may be applied to distinguish *BRCA1* carriers from non-carriers.

Prediction of edema using pharmacogenomic analysis in a clinical trial of CML patients treated with

Gleevec™/Glivec®. *L.A. McLean¹, M. Dressman¹, I. Gathmann², R. Capdeville², M.H. Polymeropoulos¹.* 1) Novartis Pharmaceuticals Corporation, Gaithersburg, MD; 2) Novartis Pharma AG, Basel, Switzerland.

Gleevec™/Glivec® (imatinib, STI571), an inhibitor of the bcr-abl tyrosine kinase, has been shown to be highly effective in the treatment of chronic myelogenous leukemia (CML). However, a common adverse event associated with Gleevec™ treatment is that of fluid retention, particularly periorbital and other peripheral edema, which occurs in approximately 50% of patients. In an effort to develop predictive markers of edema, we evaluated blood samples collected at baseline (pre-treatment) for 105 CML patients enrolled in a Phase III clinical trial that were subsequently treated with Gleevec™. Patients were identified as having edema if they had at least one occurrence of peripheral edema or other fluid retention event following treatment with Gleevec™ for a minimum of six months. Gene expression data for more than 12,000 genes were generated from each blood sample using Affymetrix oligonucleotide microarrays. Using a subset of the patients (the predictor set), we utilized a leave-one-out strategy to identify a set of predictor genes that could differentiate between those patients who developed edema and those that did not. A second, independent subset of patients (the test set) was utilized for validation of the findings. Overall, the set of predictor genes was able to correctly predict the development of edema in 89% of all the edema cases (40 of 45). To determine the relative risk of developing edema, we compared an individual's expression of the predictor genes to the mean expression profiles from the predictor set. Patients that displayed an expression pattern that was more similar to the mean Edema profile and less like the mean No Edema profile were found to be 7.2 times more likely to develop edema (95% confidence interval: 2.4 - 21.4; $p < 0.001$). Application of these findings could provide a useful tool in the clinical setting.

Genomic profiling of primary breast cancers and cell lines using array CGH. *T.L. Naylor¹, J.D. Greshock¹, S.H. Cleaver¹, B.J. Baxter¹, A. Margolin¹, M.L. Liebman¹, R. Wooster², P.A. Futreal², M.R Stratton², B.L. Weber¹.* 1) AFCRI, University of Pennsylvania, Philadelphia, PA; 2) Wellcome Trust Sanger Institute, Hinxton, UK.

Previous strategies to identify oncogenes and tumor suppressor genes have been limited by absence of the complete human genome sequence and methods for rapid, genome-wide analysis. Array comparative genomic hybridization (aCGH) measures relative copy number by competitive differential hybridization of labeled genomic DNA to DNA microarrays. The use of densely-spaced, sequence-mapped BACs for aCGH provides a high-throughput whole genome approach to identifying genes amplified or deleted in cancers. Thus aCGH circumvents many previous limitations in identifying oncogenes and tumor suppressor genes. We have constructed arrays of 5000 human BAC clones with 1 Mb average resolution. We have characterized 30 invasive breast carcinomas and 10 breast cancer cell lines using these arrays and have developed software to analyze these data. All cell lines have been genotyped at 10 Mb resolution (or greater) across the genome (Cancer Genome Project, Wellcome Trust Sanger Institute). Genotyping data correlate closely with the aCGH profile, with the aCGH data providing additional mapping information. All primary tumors were verified as >80% tumor. We identified recurrent copy number gains in primary tumors and cell lines on chromosomes 1, 8, 17, 18 and 20 that do not contain known oncogenes. These regions range from chromosome arm duplication to complex amplification of a region represented by a single BAC. Single copy losses are less frequent than gains overall and less frequent in primary tumors than cell lines. We identified recurrent single copy losses on chromosome 6 and 8 in the primary tumors that do not contain known tumor suppressor genes. To date, homozygous losses have been seen only in cell lines. We are expanding the number of primary tumors analyzed by aCGH and identifying genes located within the regions of gain/loss. We also are incorporating histopathology and outcome data into the analysis, to evaluate correlations between genomic profiles and clinical characteristics.

Monitoring telomere sequence content in sub-nanogram quantities of DNA from primary stem-like cells. *R.D. Learish¹, G. Coppola², R.F. Bulleit¹, M. Kent-First¹*. 1) Cellular analysis, Promega Corporation, Madison, WI; 2) University of Napoli, Department of Animal Science and Food Inspection, 80055 Portici, Italy.

Studies of telomere length have been carried out in diverse areas of research such as stem cells, aging, cancer, and animal cloning. However, current methods to measure telomeres are cumbersome and require microgram quantities of DNA. Using a coupled pyro-phosphorolysis/ trans-phosphorylation reaction, we have developed a sensitive assay to quantitate telomere repeat sequence content (TRSC) in a single tube or 96-well format. The standard reaction uses a telomere-specific oligonucleotide probe to interrogate nanogram quantities of DNA without PCR amplification and is coupled to the luciferase enzyme reporter system. Primary neural progenitor cells were isolated from rat embryos and cultured in media containing 1% serum and growth factors until senescence at 30-50 days, and DNA was isolated at various time points. Southern blot analysis showed decreasing telomere restriction fragment length (TRFL) corresponding with time in culture. This observation correlated with a loss in TRSC using 10 ng aliquots of the same DNA samples in the new assay system. We subsequently tested the sensitivity of the new method using limiting amounts of genomic DNA from cultured cells, primary neural progenitor cells, or bovine blastocysts. The DNA was isolated using a magnetic resin and eluted into an appropriate buffer. Preliminary results indicate the sensitivity limit for rodent or bovine cells is approximately 300 pg of DNA-- the equivalent of approximately 100 cells. This raises new possibilities in basic and clinical research, such as monitoring global changes to telomeres in blastocysts, small colonies expanded from single cells in vitro, or limiting numbers of cells isolated by FACS.

Genotype-phenotype correlations for presenile cataracts in neurofibromatosis 2. *M.E. Baser¹, J.M. Friedman², H. Joe³, A.J. Wallace⁴, R.T. Ramsden⁵, D.G.R. Evans⁴.* 1) Los Angeles, CA, U.S.A; 2) Department of Medical Genetics, University of British Columbia, Vancouver, B.C., Canada; 3) Department of Biostatistics, University of British Columbia, Vancouver, B.C., Canada; 4) Department of Medical Genetics, St. Mary's Hospital, Manchester, U.K; 5) Department of Otolaryngology, Manchester Royal Infirmary, Manchester, U.K.

Neurofibromatosis 2 (NF2) is an autosomal dominant disease that is characterized by nervous system tumors and other abnormalities. Genotype-phenotype correlations have been studied for NF2-related tumors, but have not been evaluated for non-tumor manifestations of NF2, the most common of which is presenile cataracts. We used data from the United Kingdom NF2 registry to examine genotype-phenotype correlations for cataracts in 261 people from 191 families (160 new mutations and 101 inherited cases) who had been screened for constitutional *NF2* mutations using SSCP. Cataracts were evaluated by slit lamp. There were 82 people with nonsense or frameshift mutations (including 15 known somatic mosaics), 50 with splice-site mutations, 16 with missense mutations, 32 with large deletions, and 83 with unidentified mutations. Logistic regression was used to calculate relative risks (RR). The RR of cataracts was not significantly associated with age at diagnosis of NF2 or current age. People with nonsense or frameshift mutations were the reference group in comparisons between types of mutations. In new mutations, the RR of cataracts in people with splice-site mutations was 0.94 (95% confidence interval (CI), 0.31-2.80); with missense mutations, 0.96 (95% CI, 0.14-6.47); with large deletions, 0.39 (95% CI, 0.07-2.14); with somatic mosaicism, 0.29 (95% CI, 0.07-1.17); and with unidentified mutations, 0.30 (95% CI, 0.13-0.68). In inherited cases, the RR of cataracts in people with splice-site mutations was 0.42 (95% CI, 0.13-1.29); with missense mutations, 0.18 (95% CI, 0.03-1.06); with large deletions, 0.60 (95% CI, 0.18-1.91); and with unidentified mutations, 0.46 (95% CI, 0.12-1.84). In future work, we will evaluate age at diagnosis of cataract, which may be a more sensitive measure than presence of cataract.

DNA REPAIR GENE POLYMORPHISMS AND DNA-ADDUCT LEVELS IN BLADDER CANCER. S. POLIDORO^{1, 2}, S. GUARRERA^{1, 2}, L. DAVICO², S. CARTURAN¹, M. PELUSO³, A. MUNNIA³, A. PIAZZA¹, P. VINEIS², G. MATULLO^{1, 2}. 1) Dip di Gen, Biol e Bioch, Università di Torino; 2) Serv di Epidemiol dei Tumori, Dip di Sc Biomediche e Onc Umana, Università di Torino; 3) CSPO, Lab di Biol Mol, Firenze.

The repair of DNA damage has a key role in protecting the genome of the cell from the insults of cancer-causing agents. In industrialized countries cancer of the bladder is the fifth most common cancer in men. Although development of bladder cancer is associated with exposure to tobacco (about 50%) and occupational exposure (about 20%), only a proportion of exposed individuals will develop cancer, suggesting that there is a genetic predisposition. Polymorphisms in several DNA repair genes have been identified; however the impact on repair phenotype has not been elucidated. We analyzed the relationship between 9 DNA repair gene polymorphisms belonging to different repair pathways (XRCC1-28152A/G, XRCC1-26651A/G, XRCC1-26304T/C, XPD-35931-A/C XRCC2-31479G/A, XRCC3-18067C/T XRCC3-17893A/G, ERCC4-30147A/G, PCNA-6084G/C) and DNA damage as measured by WBC 32P-postlabeling DNA-adduct levels in about 225 individuals with bladder cancer and 175 controls. No significant difference has been observed in the genotype distribution between cases and controls for all the polymorphisms, except for XRCC3-18067C/T ($p=0.008$). Significant differences exist between cases and controls for DNA-adduct levels (0.47 ± 0.04 vs 0.20 ± 0.03 , $p<0.001$) and smoking ($p<0.00001$, 89.4% vs 55.3%, respectively). Notwithstanding we have not observed a significant difference in genotype distributions and DNA-adduct levels between smokers and non-smokers, we have found an interesting higher DNA-adduct level in individuals with XRCC1-R399H GG-AG vs AA genotypes (0.49 ± 0.05 vs 0.34 ± 0.07 , $p=0.15$; maximum values are 2.5 and 0.9, respectively) in smoker cases but not in non-smoker cases. No difference in DNA-adduct levels has been observed between genotypes in smokers and non-smokers controls. Our results mainly suggest XRCC3 involvement as a bladder cancer genetic risk factor, being possibly relevant for risk assessment. Work is in progress to analyze additional polymorphisms in the same and other genes, including linkage disequilibrium data.

Microarray Analysis of Disease Relapse in Childhood Acute Lymphoblastic Leukemia (ALL). *K.M. Brown¹, R. Pillai³, E. Perez², G.H. Reaman², D.A. Stephan¹.* 1) Research Center For Genetic Medicine, Children's National Medical Center, Washington, DC; 2) Department of Hematology and Oncology, CNMC, Washington, DC; 3) Genomic Collaborations Group, Affymetrix Inc., Santa Clara, CA.

Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy and the second leading cause of deaths from childhood cancer in the United States. Over the past four decades, the fraction of children whose treatment results in long-term, disease free survival (LTDFS) has increased from less than 5% in 1960 to approximately 70% in 1994. Despite this progress, approximately 900 children with ALL relapse every year in the United States. Treatment of patients who relapse is significantly more difficult than initial treatment of ALL, in large part because relapsed leukemia cells are much more likely to be resistant to one or more chemotherapy agents. Currently, salvage therapy for relapsed disease is only able to provide LTDFS for about 40% of these children. We hypothesize there is a common mechanism underlying therapy resistance (i.e. relapse) in ALL. Identification of genes mediating therapy resistance could 1) facilitate design of novel, targeted therapy protocols, and 2) identify patients with high risk of relapse at the time of diagnosis for targeted and/or intensified therapy protocols. To this end, we expression profiled bone marrow samples from 25 similarly-treated patients with relapsed ALL using Affymetrix oligonucleotide arrays. This collection includes samples from both the time of diagnosis and relapse for each patient. Expression profiling of these diagnosis/relapse pairs on the Affymetrix U95Av2 chip (~12,600 genes) and subsequent permutation testing based on the paired t-test statistic has identified several genes whose expression is consistently different within pairs of diagnosis and relapse samples. We are currently validating these genes on an independent set of diagnosis/relapse pairs at both the RNA and protein levels. Additionally, we will use both expression profiling and flow cytometry on an independent set of diagnosis samples to determine whether differences in expression of these genes detectable at the time of diagnosis correlate with risk of disease relapse.

The Cancer Genetics Network: Recruitment Results and Pilot Studies. *H. Anton-Culver^{1, 2}, A. Ziogas^{1, 2}, D. Finkelstein², C. Griffin², J. Hanson², C. Isaacs², C. Kasten-Sportes², G. Mineau², P. Nadkarni², J. Potter², B. Rimer², J. Schildkraut², L. Strong², B. Weber², D. Winn², R. Hiatt², S. Nayfield².* 1) University of California, Irvine, CA; 2) The Cancer Genetics Network.

In 1998 the National Cancer Institute established the Cancer Genetics Network (CGN) to support collaborative investigations into the genetic basis of cancer susceptibility, explore mechanisms to integrate this new knowledge into medical practice, and identify ways of addressing the associated psychosocial, ethical, legal, and public health issues. Eight recruitment centers and an Information Technology Group (ITG) were established. Four centers use population-based cancer registries to contact and enroll patients and their family members. In the four clinic-based centers, physicians and health care professionals directly refer patients to CGN Centers. The ITG is responsible for developing and maintaining a registry and providing the statistical and computer informatics infrastructure necessary to perform the investigations. This resource is available to approved researchers and can provide clinical, environmental and family cancer history data on CGN enrollees, and access to the patients and their families for special studies. The CGN is conducting a variety of pilot studies: Ovarian Cancer Screening, Inherited Colon Cancer Among Sibling Pairs, Families with Prostate Cancer, Genetic and Environmental Modifiers of Cancer Risk Among Women With BRCA1/2 Mutations, Comparison of Models to Estimate Risk of Being a BRCA1/2 Carrier, Factors Affecting Participation Rates in the CGN, and the Breast Screening Study. As of May 2002, the CGN contained data on 15,045 participants and 241,948 family members. The distribution of participants in the CGN by cancer site is: prostate (2,823), breast (4,588), melanoma (533), lung (217), colorectal (1,074), ovarian (477), and other (3,489). Many of the families have familial or hereditary cancers, including 2,331 breast cancer families, 942 ovarian cancer families, 857 prostate cancer families, and 349 colon cancer families. The CGN is now a rich resource for collaborative studies on cancer genetic susceptibility, translational research and behavioral research.

BRAF and RAS mutations in human lung cancer and melanoma. *M.S. Brose^{1,2}, P. Volpe², M. Kumar², I. Rishi², E. Einhorn³, R. Gerrero¹, M. Herlyn⁴, J. Minna⁵, R. Wooster⁶, M.R. Stratton⁶, P.A. Futreal⁶.* 1) Dept Med, Hematology/Oncol, Hosp Univ Pennsylvania, Philadelphia, PA; 2) Abramson Family Cancer Research Institute, Philadelphia, PA; 3) Philadelphia VA Medical Center, Philadelphia, PA; 4) The Wistar Institute, Philadelphia, PA; 5) University of Texas Southwestern Medical Center, Tx; 6) The Wellcome Trust Sanger Institute, Hinxton, UK.

The relationship between signaling pathways and cancer is a focus of investigation because of the role of these pathways in malignant transformation and their potential as therapeutic targets. The RAS-RAF-MEK-ERK-MAP kinase pathway is one such pathway. Activating mutations in BRAF have recently been identified in 70% of melanomas as part of a genome-wide search for cancer-related mutations. BRAF mutations also were found in several other human primary cancers, including lung carcinomas. In order to extend the observation on BRAF mutations, we screened 150 adenocarcinomas and 100 squamous cell carcinomas of the lung for mutations in the kinase active site (exon 15) and glycine loop (exon 11) using capillary-based heteroduplex analysis. We are screening the same samples for the most common mutations in codon 61 of NRAS and codon 12 of KRAS, in order to determine whether mutations in BRAF occur in combination with other mutations in the same pathway, as suggested by the initial observation. We also have screened 50 primary melanoma and melanoma cell lines. To date we have identified mutations in BRAF in 7% of squamous cell carcinomas and confirmed the previous observation of BRAF mutations in more than 50% of primary melanomas. Analysis is still ongoing and final results will be presented. Mutations in BRAF may explain the increase in active (phosphorylated) forms of MEK and ERK in lung carcinomas present in a significant number of lung cancers in the absence of RAS mutations. In addition, mutations in BRAF also may be important in combination with RAS mutations, challenging the previous paradigm that only one defect in a molecular pathway is relevant in carcinogenesis.

A novel transcription factor, FTCTF, is fused to PPAR γ in follicular thyroid carcinoma. *W.O. Lui¹, T.G. Kroll², I. Leibiger¹, J. Liden³, B. Leibiger¹, S. Thoppe¹, A. Höög⁴, L.O. Farnebo⁵, J.A. Fletcher⁶, C. Larsson¹.* 1) Dept Molecular Medicine, Karolinska Hosp, Stockholm, Sweden; 2) Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, USA; 3) Dept of Biosciences, Huddinge, Sweden; 4) Dept of Oncology-Pathology, Karolinska Hosp, Stockholm, Sweden; 5) Dept of Surgical Sciences, Karolinska Hosp, Stockholm, Sweden; 6) Dept of Pathology, Brigham and Women's Hosp, Boston, USA.

Both papillary thyroid carcinoma (PTC) and follicular thyroid carcinoma (FTC), derive from the thyroid follicular epithelial cells. However, they display distinct differences in biological and clinical behaviors. Interestingly, a common mechanism of fusion oncogene resulting from chromosomal rearrangements has been observed in these tumors. In PTC, somatic rearrangements commonly involve RET and NTRK1 genes, in which the tyrosine kinase domain of the gene is fused to the N-terminus of another gene. To date, only one fusion oncogene is reported in FTC, involving PAX8, which encodes a paired domain transcription factor essential for thyroid development, and the peroxisome proliferator-activated receptor gamma (PPAR γ) gene. In the previous study, it was suggested that the abrogation of normal PPAR γ function may play a critical role in thyroid follicular tumorigenesis.

In this study, we cloned a second PPAR γ rearrangement, from our previously reported FTC involving t(3;7). We identified a novel transcription factor, which is fused to the N-terminus of PPAR γ gene, and demonstrated the expression of the fusion product in RNA and protein levels. In addition, we characterized the expression distributions of the novel gene identified. Finally, we compared the transcription program regulated by this fusion gene and the PAX8-PPAR γ .

IDENTIFICATION OF CANDIDATE TRANSCRIPTS AT CHROMOSOMES 10q25-q26 AND 9p21 IN ENDOMETRIAL CANCER (EC) AND MALIGNANT MELANOMA (MM). A. Manca¹, P. Baldinu¹, A. Cossu², M.P. Satta¹, M.C. Sini¹, S. Tore¹, M. Casula¹, M. Pisano¹, C. Rozzo¹, S. Dessole³, A. Pintus², A. Lissia², A.A. Carboni², F. Tanda², G. Palmieri¹. 1) Istituto Genetica Popolazioni, C.N.R., Alghero; 2) Istituto di Anatomia Patologica, University of Sassari; 3) Ostetricia e Ginecologia, University of Sassari; Italy.

Loss of heterozygosity (LOH) at chromosomes 10q25-q26 and 9p21 has been reported in EC and MM, respectively. We previously identified two consensus regions of LOH: one, between D10S610 and D10S542 markers in EC; and the second, between D9S1748 and D9S126 markers in MM. To assess the existence of candidate gene(s) in these regions, bioinformatic approaches allowed to identify ESTs, which were confirmed by both RT-PCR assays and Northern blot hybridization on tissue mRNAs. RACE method was used to further extend the cDNA ends. Germline and tumor DNA from patients was used for mutation analysis. In EC, a 160 kb consensus region of allelic deletion between D10S1236 and WIAF3299 markers was defined and a putative transcription unit was predicted by computer-assisted analysis. Exons were confirmed by RT-PCR analysis and candidate proteins were also predicted. RNA expression analysis revealed at least two main transcripts (about 1.8 and 4.8 kb). Candidate transcripts share the first three exons with different genomic location and length of the fourth exon. Mutation analysis of the first three exons identified a nucleotide substitution in 1/9 (11%) tumor DNAs at exon 2 and in none of 102 normal controls. Additional cases are being investigated in order to define the role of such transcripts in EC. In MM, the allelic deletion at the D9S171 locus was firstly confirmed by both PCR analysis on primary tumor cell lines from MM patients (D9S171 was found homozygously deleted) and FISH analysis on MM tissue sections [a single signal was detected in 2/19 (11%) MM samples using a PAC clone corresponding to D9S171]. Five candidate ESTs between D9S962 and D9S1679 markers were isolated through bioinformatic approaches and transcription has been confirmed by RT-PCR; full-length cDNAs are being defined. Work was funded by Regione Autonoma Sardegna and A.I.R.C.

APC D1822V and the risk of colorectal cancer. *B.L. Niell¹, G. Rennert², L.P. Tomsho¹, J.D. Bonner¹, S.B. Gruber¹.*
1) Depts Int Med/Epidemiol, Univ Michigan, Ann Arbor, MI; 2) Technion University, Haifa, Israel.

Low penetrance colorectal cancer (CRC) susceptibility alleles may contribute significantly to the remaining risk not explained by other known risk factors. *APC* D1822V is a common SNP that has recently been suggested as a candidate susceptibility allele that reduces the risk of colorectal cancer in specific risk groups. We investigated the relationship between D1822V and colorectal cancer in the Molecular Epidemiology of Colorectal Cancer (MECC) study, a large population based case-control study of colorectal cancer in northern Israel. Eligible cases for this planned, interim analysis included 715 individuals diagnosed with incident CRC in northern Israel between March 1998 and March 2001. Controls were 715 age, sex, ethnicity, and clinic-matched individuals without CRC. Genotyping for D1822V was performed by allele specific oligonucleotide hybridization. Conditional logistic regression was performed to adjust for potential confounders. The D1822V allele frequency was 24% in the control population, and homozygous valine/valine individuals accounted for 6.2% of the control population. No strong associations were found between colorectal cancer and D1822V heterozygous genotype (OR= 0.9, 95% CI 0.7-1.1) or homozygous variant (valine/valine) genotype (OR= 0.9, 95% CI 0.5-1.5) when compared to homozygous (aspartic acid/aspartic acid) genotype in matched, unadjusted analyses. Adjustment for family history of colorectal cancer and I1307K (in addition to age, gender, and ethnicity) did not measurably influence the results. Codon 1822 is located between the fourth and fifth b-catenin binding and regulatory domains, but no known functional consequences of D1822V are known to date. These data suggest that D1822V is unlikely to be a strong susceptibility allele for colorectal cancer, although further analysis is required to evaluate whether there is any evidence of effect modification of environmental risk factors by D1822V.

Differential Gene Expression Between Tumorigenic and Non-tumorigenic Hybrid Clones of Human Ovarian Carcinoma Cell Line SKOV-3. *N. Wang¹, R. Abeysinghe¹, L. Li¹, B. Kim², R. Miller³, J. Xu¹.* 1) Dept Pathology, Univ Rochester Sch Medicine, Rochester, NY; 2) Department of Microbiology and Immunology, Univ Rochester Sch Medicine, Rochester, NY; 3) Department of Obstetrics and Gynecology, Univ Rochester Sch Medicine, Rochester, NY.

We have previously reported that the introduction of chromosome 11 suppresses tumorigenicity in the ovarian cancer cell line SKOV-3, indicating the presence of tumor suppression gene(s) (TSG) on chromosome 11. To identify these TSG, we performed cDNA microarray analysis on tumorigenic and non-tumorigenic hybrid clones derived from the parental cell line SKOV-3. Of the 30,000 genes investigated by microarray, 274 (0.9%) showed differential expression profiles between non-tumorigenic, slow-growing tumorigenic and fast-growing tumorigenic cells. Only 7 of the 274 differentially expressed genes, LDHA, KIAA 0750, FKBP2, CRYAB, THY1, THY1 co-transcribed and cDNA FLJ23593 (HS287749) are located on chromosome 11 in three minimal regions with high frequency of LOH in ovarian cancers. Five of these 7 genes, LDHA, CRYAB, THY1, THY1 co-transcribed and cDNA FLJ23593 (HS287749), were also analyzed by real time PCR which confirmed a suppressed expression of these genes in tumorigenic clones/cell lines. Among the 268 non-chromosome 11 differential expressed genes, CAV1, HPAC3, IGFBP7, and SPARC were putative tumor suppressor genes (PTSG) identified previously by others. The up-regulation of these genes via chromosome 11 transfer suggests the presence of a regulatory gene(s) on chromosome 11 for these PTSG. Indeed by abolishing the Thy-1 expression in one of the non-tumorigenic clone 11(c)9-8, through the transfection of Thy-1 antisense results in a significant suppression of the expression of both CAV1 and CDH6 indicating the regulating role of Thy-1 on these two genes. In summary, our findings provide fundamental information to better understand the molecular basis of ovarian carcinogenesis.

Analysis of mRNA Levels in Prostate Cancer; An Investigation of Endoglin as a Prognostic Marker of Tumor Progression. *C.L.T. Clelland¹, C. Bancroft¹, J.D. Clelland²*. 1) Dept Physiology & Biophysics, Mount Sinai Hospital, New York, NY; 2) Dept Movement Disorders and Molecular Psychiatry, Nathan Kline Institute for Psychiatric Research, Orangeburg, NY.

Endoglin, also called CD105, is a homodimeric membrane glycoprotein component of the transforming growth factor beta receptor complex, primarily associated with human vascular endothelium. Endoglin is also found on bone marrow proerythroblasts, activated monocytes, and lymphoblasts in childhood leukemia. Murine studies have shown that endoglin is critical for angiogenesis, and reduced levels of functional endoglin protein, caused by mutations in the human gene, result in Hereditary Hemorrhagic Telangiectasia (HHT), an autosomal dominant vascular dysplasia leading to telangiectases and arteriovenous malformations of skin, mucosa, and viscera.

Endoglin is highly expressed on endothelial cells during angiogenesis in tumors, with much lower expression detected in normal blood vessels. Significantly, vascular density has been proposed as a promising prognostic marker in a variety of tumor types and recently a study involving immunohistochemical staining of the endoglin protein in prostate tumor specimens, documented a strong association between the density of endoglin stained tumor microvessels and Gleason score, tumor stage, presence of metastasis at diagnosis, and cancer specific survival. We have further investigated the use of endoglin as both a diagnostic and prognostic marker of prostate cancer. In this preliminary study we have measured mRNA levels of the endoglin gene in leukocytes obtained from prostate cancer patients and healthy control subjects. Here we describe the preliminary findings of our study, and discuss their implications for staging and prognostic testing of patients with prostate cancer.

The Breast Cancer Family Registry:1997-2002. *J.C. Beck¹, J.L. Hopper², E.M. John³, J.A. Knight⁴, S.L. Neuhausen⁵, R.T. Senie⁶, I.L. Andrulis⁴, H. Anton-Culver⁷, S. Buys⁵, D. West³, A. Whittemore³, M.B. Daly⁸, D. Seminara⁹.* 1) Coriell Inst Medical Research, Camden, NJ; 2) U. of Melbourne, Melbourne, Australia; 3) Northern California Cancer Center, Union City, California; 4) CancerCare Ontario, Toronto, CA; 5) U. of Utah, Salt Lake City, Utah; 6) Columbia U., New York, NY; 7) U. of California, Irvine, CA; 8) Fox Chase Cancer Center, Philadelphia, PA; 9) National Institute of Health, NCI, DCCPS, EGRP, Bethesda, MD.

Breast cancer etiology is complex, involving interplays of genetic and environmental factors. Genetic risk may be due to mutations in high-risk genes and to common genetic variants in metabolic pathways. Complementary study designs are necessary to identify and characterize breast cancer susceptibility genes and address genetic and environmental modifiers of risk. The goals of the international Breast Cancer Family Registry (B-CFR) are to: characterize breast and ovarian cancer susceptibility genes for genotype frequency and penetrance; evaluate the roles of genetic and environmental modifiers of penetrance; map new susceptibility genes and assess putative candidate genes. The B-CFR enrolls population-based (PB) families, clinic-based (CB) multiple-case families and population-based controls (PC). Dual ascertainment allows flexibility in study design. Family history of breast and other cancers is collected and validated. A risk factor and diet questionnaire is completed, blood or mouthwash samples and tissue blocks are collected and lymphoblastoid cell lines are established. Anonymized data are stored/managed by a Central Data Base. As of 5/02, 5240 PB families, 2,575 CB families and 2379 PC have enrolled, 68% white, 16% Ashkenazi, 16% minorities. 236 CB and 622 PB families with 3 or more cases of BC, 731 early-onset cases (<36 years), 1,056 cases with multiple primary breast cancer, 557 affected sister pairs have enrolled. Testing of 8,449 participants for BRCA1 mutations and 6,650 for BRCA2 has identified 700 carriers of deleterious mutations. Strengths of the B-CFR are: focus on family data; dual ascertainment; emphasis on Ashkenazi and minorities recruitment and quality and completeness of data and biospecimens. For access information go to: <http://epi.grants.cancer.gov/BCFR/index.html>.

Program Nr: 439 from 2002 ASHG Annual Meeting

Linkage Analysis on Prostate Cancer Aggressiveness. *S.L. Slager, D.J. Schaid, J.M. Cunningham, S.K. McDonnell, A. French, B.J. Peterson, A.F. Marks, S. Hebring, B. Koch, S. Anderson, S.N. Thibodeau.* Mayo Clinic, Rochester, MN.

Prostate cancer is the most common cancer among men in the United States, yet the disease etiology is still not fully understood. Several studies have demonstrated that genetics plays an integral role in its etiology, but only recently has the genetic basis of prostate-cancer aggressiveness been investigated, with several putative regions identified on chromosomes 5q, 7q, and 19q (Witte et al., *AJHG* 67:92, 2000). Tumor aggressiveness can be assessed by a pathologist using the Gleason score, which is a cancer grading system based on architectural pattern and ranges between 2 (low grade) and 10 (high-grade). To confirm the findings of Witte et al., we analyzed 190 brother pairs from 161 families affected by prostate cancer using the Haseman-Elston linkage analysis method. With Gleason score as the quantitative trait, we regressed the squared trait difference, as well as the mean-corrected cross product, on the estimated proportion of alleles shared identical by descent at each marker position. Our analysis reproduced the results of Witte et al. for chromosome 19. Specifically, we found a significant association ($P=0.0001$) between marker D19S902 (at 67 cM) and the squared trait difference. However, the associations on chromosomes 5 and 7 were not as strong. On chromosome 5, the most significant result was at marker D5S436 ($P=0.025$), and on chromosome 7, it was at marker D7S516 ($P=0.036$). Full genome wide results will be presented. Our findings and the findings of Witte et al. strongly suggest that chromosome 19q harbors a susceptibility locus for prostate cancer aggressiveness.

Establishment of the physical map in the 17p11.2 critical region for BHD gene discovery. *V. Matrosova¹, M. Nickerson¹, M. Warren¹, N. Sharma¹, L. Schmidt², B. Zbar¹.* 1) Lab ImmunoBiology, NCI/NIH, Frederick, MD; 2) SAIC-Frederick, Inc., Frederick, MD.

Birt-Hogg-Dub syndrome (BHD), an inherited autosomal genodermatosis, characterized by benign tumors of the hair follicle (fibrofolliculomas), has been associated with renal neoplasia, lung cysts and/or spontaneous pneumothorax. Previously we localized the BHD gene locus to chromosome 17p11.2 by linkage analysis in several BHD kindreds. Chromosome 17p11.2 is an unstable genomic region harboring a number of low-copy repeats associated with several genetic disorders including Smith-Magenis Syndrome. The gene density (1 gene/37 kb) in this region is much higher than the average in the human genome (1 gene/90 kb). Genome assemblies using NCBI (<http://www.ncbi.nlm.nih.gov>), UCSC Genome Browser (<http://genome.ucsc.edu>) and Celera (<http://www.celera.com>) genome databases, and our FISH mapping data revealed discrepancies among available information. Our map agrees with the UCSC Genome Browser genomic organization (August 6, 2001 release) in the 17p11.2 region between D17S1857 and AGAT100 markers. Comparison of this region to other genomic assemblies reveals that Celera's contig is reversed in orientation and the public NCBI contig is improperly assembled. In this study using overlapping BACs, we combined molecular biological technique and cytogenetic analysis to make our map more reliable. Fourteen BACs and PACs were used to provide coverage throughout the region of interest. The analysis of 12 polymorphic markers and 11 known and predicted gene PCR products from the BHD interval was combined with fluorescent in situ hybridization (FISH) of BAC/PAC probes on interphase nuclei and metaphase spreads from human B-lymphocytes. We have generated a physical map of the BHD 1.8 Mb critical interval flanked by D17S1857, a polymorphic marker from the distal region and AGAT100, a proximal marker. The correct genomic organization of the BHD syndrome region is critical to narrow the field of search for candidate genes. A promising candidate gene was identified for mutation analysis in BHD patients.

Promoters of DAPK, CDH1 and RARB Genes are Frequently Methylated in Cervical Carcinoma and Show no Association with Clinical Outcome or Microsatellite Instability. *G. Narayan¹, H.A. Pulido¹, S. Koul¹, A. Schneider², V.V.V.S. Murty¹.* 1) Department of Pathology, College of Physicians & Surgeons of Columbia University, New York, New York; 2) Department of Obstetrics & Gynecology, Friedrich Schiller University, Jena, Germany.

Promoter hypermethylation is believed to play an important role in tumor suppressor pathways in cancer. Although much is known about the etiology and treatment of cervical carcinoma (CC), the role of epigenetic alterations in the multi-step pathway of cervical tumorigenesis is poorly understood. In our goal to understand the molecular basis of CC, we performed a comprehensive promoter hypermethylation profiling of 12 genes and microsatellite instability (MSI) in 49 primary CCs. Promoter methylation of at least one locus was found in 37 of 49 (75.5%) cases. The Death-Associated Protein Kinase (DAPK1) (55.1%), Cadherin 1(CDH1) (34.7%) and Retionic Acid Receptor Beta (RARB) (18.4%) genes were frequently methylated. Seventeen of 45 (37.8%) tumors showed MSI. No correlation was found between promoter hypermethylation of any gene and MSI, HPV type and stage of the disease, or clinical outcome after the treatment. The frequent methylation of DAPK, CDH1, and RARB in invasive CC, in the absence of clear associations with genetic changes or clinical parameters, suggests a major role for the epigenetic inactivation of these genes in CC development.

Analysis of methylation status of *RBI* promoter in retinoblastoma. *P.S. Lai*¹, *R. Rong*², *Y.F.L. Ling*³, *T.C. Quah*¹, *P.S. Low*¹. 1) Dept of Pediatrics, National Univ Singapore, Singapore, Singapore; 2) Dept of Pharmacology, State University of New York, Syracuse, NY; 3) Singapore National Eye Centre, Singapore.

Retinoblastoma is a retinal cancer caused by loss of function of both alleles of the retinoblastoma tumor suppressor gene, *RBI*. In retinoblastoma patients, the somatic mutations arising in retinal cells or their precursor cells that have been identified include loss of heterozygosity (LOH) at the *RBI* locus on chromosome 13, small nucleotide mutations within the gene and hypermethylation of the *RBI* promoter region. Previous reports on the methylation pattern of the CpG rich island in the *RBI* promoter indicated evidence of hypermethylation in 9-13% of unilateral sporadic retinoblastoma tumors. To further investigate the role of hypermethylation as an inactivating event for loss of *RBI* expression, we examined the methylation status of *RBI* promoter by bisulfite sequencing analysis. A total of 21 unilateral sporadic tumors were initially screened for LOH and point mutations in *RBI*. Fourteen out of 21 tumors showed LOH (66.7%) and a second truncating mutation was identified in 11 out of the 14 LOH-positive tumors. In the 6 non-LOH cases, there were 3 tumors in which no mutation could be detected despite DNA sequencing of the entire coding exons and flanking introns of the *RBI* gene. These six tumors were thus subjected to bisulfite treatment and both strands of DNA were then amplified by primers specific for both methylated and unmethylated segments of the *RBI* promoter. The amplified products were then sequenced to identify the methylation status. Our results show lack of hypermethylation in all six cases suggesting that this epigenetic change may not be a major mechanism in the development of retinoblastoma in our patients.

Promoter methylation of the TSLC1 gene in advanced lung cancer. *Y. Murakami¹, T. Fukami¹, H. Fukuhara¹, M. Kuramochi¹, T. Maruyama¹, K. Isogai¹, M. Sakamoto², R.H. Reeves³.* 1) Tumor Suppression & Functional Genomics Project, Natl. Cancer Ctr. Res. Inst., Tokyo, Japan; 2) Pathology Div., Natl. Cancer Ctr. Res. Inst., Tokyo, Japan; 3) Dept. of Physiology, Johns Hopkins University School of Medicine, Baltimore, MD.

TSLC1 is a novel tumor suppressor gene in human non-small cell lung cancer (NSCLC) on 11q23.2 that we recently identified by functional complementation (1). Loss or markedly reduced expression of the TSLC1 was frequently observed in NSCLC cells, where promoter methylation was strongly correlated with loss of expression. Here, we examined methylation status of the TSLC1 gene promoter in 48 primary NSCLC tumors as well as 64 cancer cell lines derived from various organs by bi-sulfite-SSCP. Bi-sulfite-SSCP of cloned fragments with known methylation status indicated that it could clearly distinguish the fragments that were unmethylated at all 6 CpG sites adjacent to the predicted TATA box sequence from those fully or partially methylated. These analyses revealed that the TSLC1 promoter was significantly methylated in 18 of 48 primary NSCLC tumors (38%), including 10 of 27 adenocarcinomas, 7 of 14 squamous cell carcinomas, and 1 of 5 large cell carcinomas. Promoter methylation was more likely to be observed in relatively advanced tumors of stage Ib, II, III, and IV with TNM classification of pT2, T3, or T4 (17 of 36, 47%) than in those of stage Ia with pT1 (1 of 12, 8.3%). Furthermore, promoter methylation was observed in 19 of 64 cell lines (39%) derived from cancers of the lung (NSCLC and small cell lung cancer), esophagus, stomach, colon, breast, uterus and ovary as well as osteosarcoma and frequently accompanied by LOH on 11q23.2. These results suggest that alteration of TSLC1 would be involved in the progression of NSCLC tumors as well as in the formation of a various human cancers. We also propose that bi-sulfite-SSCP is a sensitive and practical method to detect the overall status of the promoter methylation of the relevant gene. (1) Kuramochi et al., *Nat. Genet.*, 27, 427-430, 2001.

Frequent epigenetic inactivation of the RASSF1A tumour suppressor gene in testicular tumours plus methylation profiling of testicular tumours and relationship to clinicopathological features. *S. HONORIO¹, A.*

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Testicular germ cell tumours (TGCTs) are rare heterogeneous neoplasms with differing histological patterns and malignant potential. Like other solid tumours, these may arise through multiple genetic/epigenetic changes in oncogenes and tumour suppressor genes (TSGs). We and others have recently shown that the 3p21.3 TSG RASSF1A is frequently inactivated in various tumour types including lung, breast and kidney. We have now investigated the role of RASSF1A inactivation in primary TGCTs. RASSF1A promoter hypermethylation was detected in 71% (17 of 24) TGCTs from the two major histological types, seminoma and non-seminoma. RASSF1A methylation was significantly less in seminomas versus non-seminomas ($P=0.0346$). We also determined the methylation status of 9 other genes known to be epigenetically inactivated in cancer. None of these genes were methylated in seminomas, whilst in non-seminomas methylation percentages were: MGMT, 47%; FHIT, 29%; APC, 24%; CDH13, 12%; CDH1, 11%; RARBeta, 5%; p16INK4A, 0%; GSTP1, 0% and DAPK, 0%. 61% of non-seminomas but no seminoma samples demonstrated promoter methylation at two or more genes ($P=0.0016$). In conclusion, we demonstrate that RASSF1A inactivation by promoter hypermethylation is the most frequent epigenetic event in TGCTs, suggesting that RASSF1A is a candidate 3p21.3 testicular TSG. Furthermore, our findings are consistent with a multistep model of pathogenesis in which RASSF1A methylation occurs early in tumourigenesis and additional epigenetic events characterize progression from seminoma to non-seminoma.

Methylation profiling of Wilms' tumour and adult renal cell carcinoma. *K.J. Wagner¹, N.V. Morgan^{1,2}, R.D. Lees^{1,3}, M.R. Morris^{1,2}, W.N. Cooper¹, R.G. Grundy^{1,4}, F. Macdonald³, T. Kishida⁵, M. Yao⁵, P. Schraml⁶, F. Latif^{1,2}, E.R. Maher^{1,2,3}.* 1) Section of Medical and Molecular Genetics, Department of Paediatrics and Child Health, University of Birmingham, Birmingham B15 2TT, UK; 2) Cancer Research UK Renal Molecular Oncology Group, University of Birmingham, Birmingham B15 2TT, UK; 3) West Midlands Regional Genetics Service, Birmingham Womens Hospital, Birmingham B15 2TG, UK; 4) Department of Paediatric Oncology, Birmingham Childrens Hospital; 5) Yokohama City University School of Medicine, Japan; 6) Institute for Pathology, University of Basel, Switzerland.

To investigate the role of epigenetic gene silencing in the pathogenesis of Wilms' tumour (WT) and adult renal cell carcinoma (RCC) we determined their methylation profile using a candidate gene approach. Thus 40 WT and up to 49 adult RCC were analysed by methylation-specific PCR for promoter methylation at *CASP8*, *CDH1*, *CDH13*, *DAPK*, *MGMT*, *p14^{ARF}*, *RASSF1A*, *p16^{INK4a}* and *RARb* in primary WT and *CDH1*, *CDH13*, *DAPK*, *MGMT*, *RASSF1A*, and *RARb* in primary RCC. WT demonstrated a high incidence of *RASSF1A* (56%), *CASP8* (43%) and *MGMT* (30%) methylation and intermediate frequencies at *p14^{ARF}* (15%), *p16^{INK4a}* (10%) and *DAPK* (11%). The frequency of *MGMT* methylation was higher in stage 1 and 2 tumours (50%) than in stage 3 and 4 tumours (17%) but this did not reach statistical significance ($p=0.06$). RCC were most frequently methylated at *RASSF1A* (25%), *DAPK* (24%), *CDH1* (16%), and *MGMT* (9%). Papillary RCC demonstrated a higher frequency of *DAPK* methylation (43%) than clear cell tumours (19%) ($p=0.14$). We have demonstrated that *de novo* promoter methylation is frequently implicated in the pathogenesis of WT and RCC but that methylation profiles differ between the two tumour types. Combining our results with data from previous studies, it appears that promoter methylation occurs frequently (>20% of primary tumours) at *CASP8*, *RASSF1A*, *H19* and *MGMT* in WT and at *RASSF1A*, *TIMP3*, *DAPK*, *CDH1* and *GSTP1* in RCC.

Mitochondrial DNA mutations in the buccal mucosal cells of smokers with lung cancer. *L.L Liu¹, P. Shields², L.J. Wong¹*. 1) Institute for Molecular and Human Genetics, Georgetown University Medical Center, Washington DC; 2) Lombardi Cancer Center, Georgetown University Medical Center, Washington DC.

Tobacco effects on buccal mucosal cell have been demonstrated in some studies. Smoking is the single greatest risk factor of lung cancer. It damages the DNA of buccal mucosal cell. The mitochondrial DNA (mtDNA) mutations scattered through coding and noncoding regions have been reported in lung cancer. The aim of this study was to determine whether mtDNA mutations could be detected in buccal mucosal cells of the patients with lung cancer and whether mtDNA mutations in buccal mucosal cells would provide an alternate source of tissue for lung cancer biomonitoring. Eight buccal mucosal cells were brushed, and their corresponding blood samples were drawn from the patients with lung cancer. The entire mitochondrial genome was amplified with 32 pairs of overlapping primers. MtDNA mutation was screened by temporal temperature gradient gel electrophoresis (TTGE) method. DNA fragments showing different banding patterns between buccal mucosal cell and paired blood mtDNA were sequenced to identify the mutations. All 8 (100%) samples had somatic mtDNA mutations. Seven of them had mutations in the hypervariable D loop region. Three buccal mucosal cells had one mutation each. Each of the remaining samples had multiple somatic mutations. Among a total of 19 somatic mutations, 7 were in mRNA (36.8%) and 12 (63.2%) in the hypervariable D loop region. All of the mRNA mutations are located in COX subunit encoding region. Fifteen DNA alterations were at the heteroplasmic state in buccal mucosal cell. The remaining 4 mutations were homoplasmic. Common 5 kb deletion of mtDNA was not detected in all samples by PCR. The high incidence of mtDNA mutations found in buccal mucosal cells in patients with lung cancer suggests that smoking causes mtDNA alterations that may play an important role in tumorigenesis of lung cancer. Further studies will be needed to determine the pathological effects of somatic mtDNA mutations and their relationship to lung cancer.

Genetic association of the UDP-glucuronosyltransferase (*UGT1A1*) gene polymorphisms with hepatocellular carcinoma in a Korean male population. C.S. Ki¹, K.A. Lee¹, J.H. Lee², S.S. Cho³, J.W. Kim¹. 1) Department of Laboratory Medicine, Samsung Medical Center, Seoul, Korea; 2) Department of Internal Medicine, Samsung Medical Center, Seoul, Korea; 3) Center for Clinical Research, Samsung Biomedical Research Institute, Seoul, Korea.

Although chronic infection with hepatitis B (HBV) or C virus (HCV) is a well-known risk factor for hepatocellular carcinoma (HCC), only about one fifth of chronic carriers are expected to develop HCC in their lifetime, suggesting that there may be an inter-individual variation in susceptibility to HCC. This study was designed to test a hypothesis that any functional polymorphisms in the genes related to lowering antioxidant capacity may be associated with genetic susceptibility to oxidative damage and HCC. Since bilirubin is a well-known physiological antioxidant and several functional polymorphisms in the *UGT1A1* gene explaining unconjugated hyperbilirubinemia have recently been reported, we performed an association analysis by comparing the genotype and allele frequencies of the *UGT1A1* gene polymorphisms between 212 male patients with HBV-associated HCC and 329 age- and sex-matched controls. Three functional polymorphisms in the *UGT1A1* gene were genotyped by direct sequencing; a -3263T/G polymorphism at the phenobarbital-responsive enhancer module, a TA repeat polymorphism (*UGT1A1**28) in a putative TATA box in the promoter region, and a 211G/A polymorphism (Gly71Arg; *UGT1A1**6) in exon 1. There was no statistical difference in the genotype and allele frequencies of the *UGT1A1* gene polymorphisms between HCC and controls. However, serum bilirubin concentrations were significantly different among controls with different *UGT1A1* genotypes ($p < 0.0001$). Based on this observation, HCC and controls were divided into two groups with low or high bilirubin genotypes. Reanalysis with these groups revealed that subjects with high bilirubin genotypes were significantly fewer in HCC than those in controls (11.7% vs. 19.1%; $c^2 = 5.19$, $p = 0.022$, OR = 1.79, 95%CI 1.05 - 3.08), which is consistent with our hypothesis. These data suggest that the *UGT1A1* gene polymorphisms may, in part, explain inter-individual susceptibility to HCC.

Novel chemotherapy resistance markers in osteogenic sarcoma. *M.B. Mintz^{1,3}, R. Sowers², R. Gorlick², D.A. Stephan¹.* 1) Dept Genetic Medicine, Childrens Hosp, Washington, DC; 2) Memorial Sloan-Kettering Cancer Center, New York, NY; 3) George Washington University, Department of Genetics, Washington, DC.

Osteosarcoma is a bone tumor that occurs predominately in adolescents and adults, with the average age of diagnosis being 15 years of age. Osteosarcoma accounts for approximately 6% of childhood tumors and is the most common malignant bone tumor in children. Current treatment for osteosarcoma involves multi-agent chemotherapy followed by limb salvage surgery. Drug resistance continues to be the major problem preventing long-term survival of many osteosarcoma patients. Tumors that do not respond to preoperative chemotherapy result in patients with poor survival rates due to the occurrence of metastases and relapse. Due to the lack of reliable markers predicting the response to chemotherapy at the time of diagnosis, risk assessment and subsequent treatment modification is not possible. The goal of this project is the identification of novel markers that are associated with poor prognostic outcome and low disease-free survival rates in osteosarcoma patients. 40 tumor samples from patients before chemotherapy treatment were studied. 20 tumor samples were obtained from patients who did not respond to chemotherapy, while the remaining 20 samples were obtained from patients who were responsive to chemotherapy. The gene expression profiles from these tumor samples was investigated through the use of using Affymetrix® U95Av2 GeneChips (~12,600 genes). Data extraction and normalization was conducted using Affymetrix® MicroArray Suite 5.0. Thereafter, genes were cropped in a systematic fashion to eliminate those that showed no expression or little variation across all of the tumors. Supervised clustering was used to identify expression correlates of therapy resistance, and these genes were expanded to encompass a pathogenic model. We are currently validating these markers on independent sample sets. We hope to begin prospective diagnostic trials as well as preclinical studies with compounds that target resistant tumors shortly.

SCREENING FOR GERMLINE MUTATIONS OF CANDIDATE GENES IN SARDINIAN CANCER

FAMILIES. *M. Colombino*¹, *G. Palomba*¹, *A. Cossu*², *M. Pisano*¹, *M.P. Satta*¹, *M.G. Sarobba*³, *A. Farris*³, *M.F. Dedola*⁴, *A.A. Carboni*², *A. Manca*², *F. Tanda*², *G. Palmieri*¹. 1) Istituto Genetica Popolazioni, C.N.R., Alghero; 2) Istituto di Anatomia Patologica, University of Sassari; 3) Oncologia Medica, University of Sassari; 4) Radioterapia, University of Sassari; Italy.

Sardinian population is genetically homogeneous and seems to be instrumental to better understand the genetic counterpart of a complex disease like cancer. To evaluate the role of disease-causing mutations in breast cancer (BC) as well as colorectal carcinoma (CRC), we screened tumor susceptibility genes (BRCA1 and BRCA2, for BC; MLH1 and MSH2, for CRC) in apparently unrelated Sardinian BC/CRC cancer families with at least three affected members. Paired normal and tumor samples from familial CRC patients were screened by PCR for microsatellite instability (MSI), followed by MLH1/MSH2 mutation screening on germline DNA. For BC, two deleterious BRCA1/2 germline mutations (BRCA2-8765delAG and BRCA1-Lys505ter) were found in 15 (23%) out of 64 BC families analyzed. BRCA2-8765delAG was detected in 13/15 (87%) BRCA1/2-positive families and demonstrated to be a founder mutation. Prevalence of BRCA1/2 mutations in BC families was significantly correlated to the total number of BC cases and increased by the presence of a) at least one case of ovarian or male-breast cancer; or b) three generations affected, or c) bilateral BC. For CRC, MSI was markedly higher in familial (18/27; 67%) than sporadic (32/193; 17%) cases among 220 patients investigated for family cancer history. To date, 1 (4%) and 10 (40%) out of 25 CRC families presented MLH1 and MSH2 germline mutations, respectively. Again, MLH1/MSH2 mutations were associated to the total number of either CRC or endometrial cancer (EC) cases in the family [7/12 (58%) families with at least 4 CRC/EC cases vs. 4/13 (31%) families with three or less CRC/EC cases]. BRCA2 and MSH2 mutations seem to be strongly correlated to cancer development in BC and CRC families from Sardinia, respectively. Presence of the above-described features should address BC/CRC patients and their families to genetic counseling and mutation analysis. Work was funded by Regione Autonoma Sardegna and A.I.R.C.

A population based study in Northern Sweden: cancer risks and mutation analysis of the *hMLH1*, *hMSH2* and *hMSH6* genes in patients with double primary tumours of the colorectum and the endometrium. *K. Cederquist*¹, *M. Emanuelsson*², *I. Goransson*², *R. Stenling*³, *I. Golovleva*¹, *H. Gronberg*². 1) Med Biosciences, Med Genetics, Umea University, Umea, Sweden; 2) Radiation Sciences, Oncology, Umea University, Umea, Sweden; 3) Med Biosciences, Pathology, Umea University, Umea, Sweden.

Hereditary non-polyposis colorectal cancer, HNPCC, is an autosomal dominant condition predisposing to cancers primarily of the colorectum and the endometrium. The aim of this study was to estimate the risk of colon and other HNPCC-associated cancers in relatives of persons with double primary (DP) tumours of the colorectum and the endometrium and to investigate the *hMLH1*, *hMSH2* and *hMSH6* mutation spectrum of the microsatellite instability (MSI) positive patients.

Family histories of cancer were obtained on 89 persons with DP cancers of the colon and the endometrium, identified through the Regional Cancer Registry at the University Hospital of Umea. The cancer risks in their 649 first-degree relatives and the MSI status of 78 proband tumours were analysed. The cancer risks were estimated in relation to MSI status and age at diagnosis in the proband (over or under 50 years) and the standardised incidence ratio (SIR) was calculated. The *hMLH1*, *hMSH2* and *hMSH6* mutation spectrum of 23 MSI positive patients was investigated by DHPLC analysis and direct sequencing.

The overall SIR was 1.69 (95% CI; 1.39-2.03). The highest cancer risk was seen in the ≤ 50 years age group (SIR=2.67), especially when MSI positive (SIR=3.17). 30 tumours (38.5%) were MSI positive. 14 MSI positive patients had sequence variants of *hMLH1*, *hMSH2* or *hMSH6*, likely affecting the function of these genes. The majority of the mutations (71%) were found in patients diagnosed before or at the age of 50. Four of the mutations detected were novel: one frameshift mutation affecting *hMSH2*, one nonsense mutation affecting *hMSH6* and two missense mutations affecting *hMLH1* and *hMSH6*. Notably, the *hMSH6* mutations were found in six different families originating from the same geographical area and therefore expected to be founder mutations.

RNASEL mutations are infrequent in hereditary prostate cancer families. *K.A. Cooney^{1,2}, K.A. Zuhlke¹, A. Griffin¹, Y.Q. Wu¹, L.P. Tomsho¹, S.B. Gruber^{1,3}, E.M. Lange⁴, H. Chen¹.* 1) Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI; 2) Department of Urology, University of Michigan Medical School, Ann Arbor, MI; 3) Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor, MI; 4) Department of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, NC.

Recently, Carpten et al. have reported the identification of mutations in the RNASEL gene occurring in two out of eight multiplex prostate cancer families with evidence of prostate cancer linkage to chromosome 1q24-25 markers (Nat Genet 30: 181, 2002). To test the hypothesis that the RNASEL gene is the prostate cancer susceptibility gene HPC1 on chromosome 1q, we examined genomic DNA from 95 affected men in 75 multiplex prostate cancer families, including 19 families with evidence of linkage to 1q24-25 markers, from the University of Michigan Prostate Cancer Genetics Project for RNASEL mutations. Direct sequence analysis of the coding region revealed four rare missense/nonsense mutations (G59S, I97L, V247M, E265X) occurring in four unrelated families. All four of the families harboring RNASEL mutations are white, and two of the families exhibited evidence of linkage to chromosome 1q24-25 markers. However, the mutation co-segregated with disease status in only one family. None of four reported rare mutations (M11, G59S, V247M, and E265X) were observed in a panel of 75 control DNA samples or in any of the 103 cases and 323 control samples from a population-based study of prostate cancer in African Americans. Taken together, our results suggest that RNASEL mutations do not completely account for the HPC1 linkage evidence and additional studies of 1q24-25 candidate genes should be performed.

Automated construction of tissue microarrays from morphologically selected sites: a powerful method for studies of genetic heterogeneity of cancer. *G.H. Hostetter¹, J. Kakareka², G. Salem², J. Hubbell¹, J. Kononen¹, U. Wagner¹, R. Cornelison¹, T. Pohida², O-P. Kallioniemi¹.* 1) Cancer Genetics Branch, NHGRI, NIH, Bethesda, MD; 2) Center Information Technology, NIH, Bethesda, MD.

Tissue microarrays (TMAs) are an invaluable tool for the rapid in situ analysis of genetic alterations and molecular targets in tumors and precursor lesions. We developed a robotic tissue arrayer to facilitate large-scale and customized TMA studies. Digital images are first scanned from hematoxylin-eosin stained tissue sections and regions are selected for punching. The arrayer automatically acquires tissue cores from pre-selected donor sites and deposits the cores in the TMA blocks. Up to 21 TMAs can be constructed at a time using a computer-controlled, motorized stage that controls the X-Y-Z movements of the punching apparatus. The robotic arrayer has been used to array up to 2250 tumors including tumor specific arrays of melanoma (352 cores), testicular germ cell tumors (532 cores), prostate tumors (240 cores) and breast tumors (300 cores). Precision and accuracy of arraying compared favorably with the manual arraying method. A centralized database permits archiving and subsequent retrieval of sites sampled in clinical samples that are heterogeneous, including precursor lesions and invasive cancer. The application of TMAs of different stages of breast cancer (normal, ADH, DCIS, and invasive cancer) in FISH analysis with Her-2, c-myc, cyclin D, and EGFR-1 oncogene probes (Vysis, Inc.) demonstrates the utility of this method for studies of tumor heterogeneity. In summary, a robotic computer-controlled tissue arraying instrument with a block marking feature was developed and applied to construct tissue arrays for studies of genetic heterogeneity of cancer.

Comprehensive assay development for Hereditary Non-polyposis colon cancer. *M.R. Hegde, D. Odenbaugh, Y.P. Weng, C.S. Richards.* Baylor College of Medicine, Department of Molecular and Human Genetics, Houston, TX.

Colorectal cancer is the second leading cause of cancer related death in the United States accounting for more than 57,000 deaths per year. While the majority of colorectal cancer is not inherited, inherited CRC accounts for up to 10% of total cases, and primarily consists of familial adenomatous polyposis (1%), or hereditary non-polyposis colon cancer (HNPCC). Hereditary non-polyposis colon cancer (HNPCC) is an autosomal dominant disorder, which is genetically heterogeneous. Mismatch repair (MMR) genes, primarily MLH1, MSH2, and MSH6, are causative of this disorder. The challenge to the identification of the HNPCC is to separate sporadic tumors from HNPCC tumors. Additionally, the large size of these causative genes makes identification of mutations both labor intensive and expensive. We have developed a comprehensive assay, which includes microsatellite instability assay (MSI), methylation assay and mutation scanning for MMR genes using denaturing high performance liquid chromatography (dHPLC). The MSI assay includes NCI recommended panel and five additional microsatellites. Hypermethylation of the MLH1 promoter is screened using a PCR-based assay. Our criteria for selection of HNPCC candidates includes family history analysis using Bethesda criteria, a high level of MSI in the tumor and absence of methylation of the MLH1 promoter prior to mutation analysis by dHPLC. The dHPLC approach is an attractive option for clinical molecular laboratories because of its high throughput capability and cost effectiveness. Our assay consists of 51 sets of primers to amplify all exons of these genes. All PCR reactions were designed for a single set of conditions and are amplified simultaneously in a 96-well format. The amplified products are analyzed by dHPLC, using the optimum temperature and variations for partial denaturation based upon each specific fragment melting profile. We anticipate no false positive results of the assay, since all positive results obtained by dHPLC are confirmed by automated sequence analysis prior to reporting. The combination of the above mentioned approaches will allow for greater sensitivity and a more directed molecular analysis.

Identification of a novel 26 kb deletion in *BRCA1* by haplotype pair analysis. *B.D. Ward, B.C. Hendrickson, C.A. Gaglio, T. Scholl.* Myriad Genetic Laboratories, Salt Lake City, UT.

The identification of intragenic rearrangements is important for a comprehensive understanding of mutations that occur in clinically important genes. We report the identification of a novel, recurrent 26 kb deletion in *BRCA1*.

An algorithm employing expectation maximization statistics was used to derive *BRCA1* haplotypes from unphased coding nucleotide polymorphism data and then identify unusual haplotypes suggestive of intragenic deletions. Data for 5911 completely anonymized samples were processed through this algorithm. Six samples were observed with a genotype that did not match pairs of known canonical haplotypes, and which possessed strong family histories of breast and/or ovarian cancer and negative clinical full gene-sequencing tests for *BRCA1* and *BRCA2*. The genotypes for these samples closely resembled a combination of the consensus haplotype and a common, non-consensus haplotype defined by eight polymorphisms in exons 9, 11, 13 and 16. In these six DNAs, the exon 16 polymorphism was homozygous for the non-consensus base, whereas the remaining polymorphisms were heterozygous. This haplotype could be explained by intragenic deletions on the consensus chromosome that include exon 16 and with upstream breakpoints 3' of exon 13.

Long range PCR results from two of the six samples produced a fragment 26 kb shorter than the expected wildtype fragment (32 kb) when amplified with primers in exon 13 and exon 21. This suggested a deletion encompassing exons 14 through 20. The deletion region was further defined using restriction mapping of the mutant fragment. The breakpoint, which resulted from inter-*Alu* recombination was completely characterized by nucleotide sequencing. Because the deletion occurred on the consensus chromosome, a group of 417 completely anonymized samples with uninformative haplotypes was assayed for this new mutation. One additional sample with the same 26 kb deletion was identified, which strongly suggests its presence in unrelated families. Finally, an allele specific PCR assay was developed and currently detects this deletion as a part of the comprehensive clinical analysis of the *BRCA* genes.

A novel method to rapidly screen for large genomic deletions or duplications in the BRCA1 gene. *G. Pals¹, P.N. Nederlof², J.J.P. Gille¹, M. Grippeling¹, R. Pruntel², F.H. Menko¹, I. Kluijt³, C. Dommering¹, S. Verhoef^{2,3}, J. Schouten⁴, L.J. van 't Veer², F.B.L. Hogervorst².* 1) Clinical Genetics, VU medical center, Amsterdam, Netherlands; 2) Family Cancer Clinic, Netherlands Cancer Institute, Amsterdam, Netherlands; 3) Department of Clinical Genetics, Academic Medical Center, Amsterdam, Netherlands; 4) MRC Holland, Amsterdam, Netherlands.

The majority of the families seeking advice at a family cancer clinic are those with familial breast/ovarian cancer. Often they are offered mutation analysis of the BRCA1 and BRCA2 genes, which is usually restricted to the analysis of PCR fragments from genomic DNA. However, a wide variety of different exon deletions and duplications in the BRCA1 gene have been described. Here, we present a simple and reliable method to detect all possible single or multiple exon deletions and duplications in the BRCA1 gene. The method, called multiplex ligation-dependent probe amplification (MLPA), is PCR based and allows the relative quantification of many different sequences in a single tube. In a collaborative study, we have tested more than eighthundred individuals in which routine DNA-diagnostic analysis did not reveal a mutation in either BRCA1 or BRCA2. This study identified 5 different mutations in this gene among which are a deletion of exon 8 and duplications of exon 21-23 and 17-19 that are not reported before. Furthermore, all identified mutations were found only once, suggesting that there is no additional high frequency founder mutation like the genomic deletions of exons 13 or 22. The MLPA test for BRCA1 proved to be a very efficient and reliable test, which can easily be implemented in the DNA-diagnostic laboratory. Furthermore, it provides a means to screen all counselees for large genomic deletions/duplications and thus improves the diagnostic mutation screening.

Tampa Bay Ovarian Cancer Study - a population-based study of BRCA1/2 in ovarian cancer. *T. Pal^{1,2}, J.P. Krischer^{1,2}, T. Holtje², J.A. Betts², J. Permuth-Wey^{1,2}, J. Fiorica^{1,2}, E. Grendys^{1,2}, J. LaPolla³, H. Arango⁴, K. Wakeley¹, M. Hoffman¹, G. Wilbanks¹, S. Nicosia^{1,2}, R. Sutphen^{1,2}.* 1) Departments of Interdisciplinary Oncology, OB-GYN, Pathology and/or Pediatrics, University of South Florida College of Medicine, Tampa, FL; 2) H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL; 3) Bay Front Medical Center, St. Petersburg, FL; 4) Morton Plant Mease Healthcare, Clearwater, FL.

BRCA1 and BRCA2 are believed to account for the majority of hereditary ovarian cancers. Current estimates of mutation likelihood among ovarian cancer patients range from 9.2% (Myriad data) to 11.7% (Ontario population data, the only published population-based data). To determine the prevalence, spectrum of mutations and genotype/phenotype correlations among ovarian cancer cases, we are conducting a population-based study of unselected incident cases of epithelial ovarian cancer in the geographic regions of Hillsborough and Pinellas counties, Florida (which includes Tampa, St. Petersburg, and Clearwater). Beginning in 2001, we have enrolled 100 women diagnosed with incident ovarian cancer, ascertained through their treating gynecologic oncologists. Medical records and tumor tissue have been reviewed and genetic counseling and DNA testing performed through full sequencing of the BRCA1 and BRCA2 coding regions and adjacent intronic base pairs. Of the first 100 women enrolled in the study, 15 (15.0%) had mutations in BRCA1 or BRCA2: 7 in BRCA1 and 8 in BRCA2. No mutations were found among the 6 cases with mucinous tumors. No mutations were found among the 5 cases with borderline tumors; thus, the mutation frequency among invasive tumors was 15.8% (15/95). These data suggest that 1) the frequency of BRCA1 and BRCA2 mutations among invasive ovarian cancer cases may be higher than previously reported, 2) previous studies may have underestimated the contribution of BRCA2 to ovarian cancer, especially mutations outside the ovarian cancer cluster region (OCCR). Preliminary data regarding risk factors, penetrance, associated cancers and tumor characteristics is being analyzed and will also be presented.

Mutation analysis of the *HCCS1* gene in medulloblastomas. F. Zhang¹, J.A. Biegel^{1, 2}. 1) Division of Human Genetics and Molecular Biology, The Children's Hospital of Philadelphia, Philadelphia, PA. 19104; 2) Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Medulloblastomas and related primitive neuroectodermal tumors (PNETs) of the central nervous system are malignant, invasive embryonal tumors with predominantly neuronal differentiation that comprise 20% of pediatric brain tumors. Cytogenetic analysis has shown that alterations in chromosome 17, particularly the loss of 17p and the formation of an isochromosome 17q, are the most common changes among this group of tumors, implicating loss or inactivation of a tumor suppressor gene in 17p. A novel tumor suppressor gene, *HCCS1*, located at 17p13.3, was recently reported to have a high frequency of mutations in hepatocellular carcinoma (HCC) samples (Zhao, X et al, Cancer Res, 61:7383-7387, 2001), whereas no alteration was found in matched noncancerous liver tissues. The purpose of this study was to determine whether *HCCS1* is a candidate tumor suppressor gene for medulloblastoma. Forty-five medulloblastoma/PNET samples and fifteen normal control cDNAs from lymphocytes or normal brain tissues, were screened for mutations within a full-length *HCCS1* cDNA using RT-PCR and sequence analysis. Three tumors had deletions of one or more exons of the *HCCS1* gene, but matched blood samples or noncancerous tissue were not available for comparison. Four polymorphisms, including the 341-427 deletion reported as a liver tumor specific mutation, were found in tumor and/or normal cDNA samples. Further studies are needed to confirm the presence of the tumor associated mutations of the *HCCS1* gene in medulloblastoma/PNET before it should be considered a good candidate for a 17p13 medulloblastoma tumor suppressor gene.

Genetic risk factors for progression to lymphoma in AIDS patients. *U. Potocnik, M.P. Martin, B. Gold, X. Gao, M. Dean, M. Carrington.* Lab. of Genomic Diversity, NCI at Frederick, Frederick, MD, USA.

The frequency of B-cell non-Hodkin's lymphomas (NHL) is high in patients infected with HIV and account for a significant fraction of HIV-related mortality. AIDS lymphomas are particularly resistant to treatment. Immune surveillance is suggested to play an important role in lymphomagenesis. Previous studies demonstrated that the deletion allele in the CCR5 chemokine receptor gene (CCR5-delta 32 allele) is associated with 3-fold lower risk of NHL. In this study we used PCR-Taqman for SNPs detection in some genes involved in immune response, multidrug resistance and tumor suppression. The killer immunoglobulin-like receptor genes (KIR) which code for one family of natural killer cell receptors were typed by PCR-SSP. For typing of KIR genes coding for Natural killer cell receptors. HLA-class I typing was performed by PCR-SSOP. The genotypes were compared between AIDS patients with NHL and AIDS patients without NHL (controls). A significant difference between the two groups was observed in the frequency of inhibitory KIR genes, particularly 2DL2 and 2DL3 alleles ($p=0.0059$). Also the distribution of HLA class I alleles differed between groups. The combination of KIR 2DL3 and HLA-Cw*0701 which serves as a ligand for KIR 2DL3 was higher in NHL patients as compared to controls. Functional MDR1 polymorphisms (G2677T and C3435T) were less frequently found in NHL patients as compared with controls though these were only at the borderline of statistical significance. We found no correlation between polymorphisms within chemokine genes CCR2 and CX3CR1 and the tumor suppressor genes BRCA1 and BRCA2 with progression to NHL. These results suggest several genes contribute to the susceptibility for NHL in AIDS patients.

Identification of a Candidate Oncogene SEI-1 within a Minimal Amplified Region at 19q13.1 in Ovarian Cancer Cell Lines. *X-Y. Guan¹, T.C.M. Tang¹, J.S.T. Sham¹, D. Xie¹, Y. Fang².* 1) Dept Clinical Oncology, Univ Hong Kong, Hong Kong, China; 2) Cancer Institute, Sun Yat-Sen University, Guangzhou, China.

High-level amplification of DNA sequence at 19q13.1-q13.2 in ovarian cancer has been frequently detected by chromosome microdissection and comparative genomic hybridization (CGH). This strongly suggests that 19q13.1-q13.2 contains putative oncogene(s) which plays an important role in the development or progression of ovarian cancer. In an attempt to verify the nature of amplification events and identify minimal amplified region (MAR) at 19q13.1-q13.2, we surveyed the amplification status in this region in four ovarian cancer cell lines that have been confirmed containing amplification at 19q13.1-q13.2. Fluorescence in situ hybridization (FISH) with 30 cosmid clones and Southern blot hybridization with 19 DNA markers within the region from D19S425 to D19S907 (about 19.5 cM) were performed. Two separated overlapping MARS, MAR1 (200 kb) and MAR2 (1 Mb), were identified at 19q13.1. MAR2 contains two candidate oncogenes AKT2 and SEI-1. Amplification and overexpression of these two genes in four ovarian cancer cell lines were confirmed by Southern and Northern blot analyses. The proliferation-related function of AKT2 and SEI-1 suggests that both genes are likely to be biological targets of amplification event at 19q13.1 in ovarian cancer and play important roles in ovarian tumorigenesis.

Correlation between NF- κ B/GM-CSF expression and functional status of the p16 melanoma susceptibility gene.

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P16 loss of expression correlates with tumor progression in sporadic melanoma and the presence of germline mutations in a subset of melanoma families suggests that its inactivation could be an early step in melanomagenesis. Alternative targets for p16 other than CDK4 have been identified: p16 also binds and inhibits NF- κ B, which regulates the transcription of a variety of genes including p53, cyclinD1 and GM-CSF. NF- κ B protein dimers are mostly detected in the cytoplasm and are bound to I κ B via ankyrin domains. Several stimuli activate NF- κ B allowing it to translocate into the nucleus. We aimed to verify whether p16-impairing alterations affect the expression of NF- κ B and dependent genes. Five metastatic melanoma cell-lines, including 1 stabilized from a familial case, were used to evaluate expression of the p16/NF- κ B/GM-CSF genes by IHC. We then analyzed NF- κ B activation by EMSA with specific antibodies (p50, p65, c-rel), and positive responsive elements, both canonical and in the GM-CSF promoter. Most of the cell lines, as compared with NHEM, exhibited constitutive activation of the NF- κ B complex, which was found to correlate with NF- κ B/I κ B expression and subcellular localization by IHC. NF- κ B expression correlated with EMSA experiments and localization of I κ B and GM-CSF expression correlated with its receptor. Methylation affected p16 expression in 3 of the 5 melanoma cell lines. These results do not rule out that alteration of p16 expression may favor constitutive transcription of different cytokines and neoplastic growth via an autocrine pathway in a tissue-specific manner. Melanoma cell lines represent useful models for studying this correlation, but in vivo expression studies on melanoma lesions can help in discriminating between different stages of progression. This study was supported by Ministry of Health Grant ICS 030.1/RF99.32 to G.B.S. The last two authors contributed equally.

A role for the DNA mismatch repair proteins Msh2 and Msh6 in the response of MEFs and keratinocytes to UVB-induced DNA damage. *S.E. Andrew¹, L. Young¹, A.C. Peters¹, L.T. Leong¹, E. Chomey², V. Tron².* 1) Dept Medical Genetics, Univ Alberta, Edmonton, AB, Canada; 2) Dept Laboratory Medicine and Pathology, Univ Alberta, Edmonton, AB, Canada.

The DNA mismatch repair (MMR) system maintains genomic stability by repairing post-replicative DNA errors. It has also been shown to repair DNA damage inflicted by exogenous agents. Recent studies have shown that MMR is also required for apoptosis in response to alkylating agents, aromatic amines and polycyclic aromatic hydrocarbons. Thus MMR may protect cells from DNA damage by sensing damage and activating repair or apoptosis pathways. The MMR protein MSH2 has previously been shown to bind to UV-induced DNA adducts and complex with the nucleotide excision repair proteins that repair such adducts, however a role for MMR post-UV has been controversial. We propose that Msh2 and Msh6 play a role in mediating cellular responses post-UV treatment. Specifically, we ask if MMR participates in the decision to repair or apoptose. We show that the MMR proteins are induced by UVB irradiation in primary keratinocytes and MEFs and this induction is at least partially p53-independent. We demonstrate in primary cells from Msh2 proficient and deficient mice that repair of UVB-induced thymine dimers is significantly impaired without functional MMR. This involvement of Msh2 in thymine dimer repair also appears to be independent of p53. Msh2^{-/-} and Msh6^{-/-} cells demonstrate increased viability following UVB irradiation. We also demonstrate reduced levels of UVB-induced apoptosis in MMR deficient fibroblasts compared to wildtype. Furthermore, in vivo experiments will determine whether Msh2 and Msh6 deficient mice display an increased susceptibility to UV-induced skin tumours, which would strongly support a role for MMR in UV-induced tumorigenesis. Thus we suggest that the MMR proteins Msh2 and Msh6 are involved in the cellular response to UVB-induced DNA damage, including thymine dimer repair, apoptosis, and tumorigenesis, affirming the possibility that the MMR system acts as a broad spectrum DNA damage sensor.

Unusual pattern of age-related somatic mutation in cell lines derived from carriers of the BRCA1 and 2 genes. *R. Das, S.G. Grant.* Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA.

Cancer, including breast cancer, is caused by the accumulation of somatic mutations in oncogenes, tumor suppressor genes and mutator genes. Most cancer-predisposing human diseases involve the loss of fidelity of DNA metabolism or repair. We have shown that these diseases are often characterized by elevated frequencies of in vivo bone marrow somatic mutation. Somatic mutation is an unavoidable consequence of cellular replication and metabolism, and the frequency of in vivo somatic mutation has been shown to significantly increase with age. We obtained immortalized lymphoblastoid cell lines from five carriers of mutations in the breast cancer predisposing BRCA1 gene, four carriers of mutations in the BRCA2 gene and nine normal individuals of widely varying age at time of blood donation. We then applied the clonogenic HPRT mutation assay to these cell lines to determine whether carrier status for these potential mutator genes affected their baseline mutation frequencies. First, we established that an aging effect indistinguishable from that observed in vivo could be demonstrated amongst the normal cell lines. The slope of the age regression indicates an increase of one new mutation per million cells every four years over lifetime. By first purging the input cell populations of pre-existing mutants we were able to demonstrate that at least two mechanisms contribute to this increase: an intrinsic increase in the mutation rate that may be related to an overall loss of cell viability, and an accumulation of mutants over time. When these same studies were repeated with cell lines established from BRCA1/2 mutation carriers, a much different pattern was evident. Cell lines from young mutation carriers had significantly higher HPRT somatic mutation frequencies than age-matched normal controls, but there was no evidence of any effect of donor age. Thus, by age 60 the frequency of somatic mutation in the cell lines from genetically normal individuals were indistinguishable from the mutation carriers. This is the first description of a distinctive cellular phenotype for carriers of BRCA1/2 mutations that could play a role in their susceptibility to breast cancer.

Qualitative and quantitative change of AIB1 in breast cancer cell lines. *P. Dai*¹, *R. Clarke*², *L.J.C. Wong*¹. 1) Inst Molec & Human Genetics, Georgetown Univ Medical Ctr, Washington, DC; 2) Department of Oncology, Georgetown Univ Medical Ctr, Washington, DC.

The amplifications of AIB1 (amplified in breast cancer) gene in breast cancer cell lines were studied by real time quantitative PCR analysis to determine their relationship to estrogen receptor status and drug (estrogen antagonist or agonist) treatment. Amplifications of AIB1 gene were found in ER positive cell lines, BT-20, ZR-75-1, BT-474, MCF-7 and T47D but not in the ER positive cell lines, BT483, MD-MB-361, MD-MB-468 and MDA-MB-330. Only MDA-MB-435 of the ER negative cell line (MD-MB-57, BT-549, MD-MB-134v, MDA-MB-231N, MDA-MB-436, HBL-100, MDA-MB-435, ZR-75-30, Hs 578T) was found to have AIB1 gene amplification. Most AIB1 amplifications occurred in the ER positive cells. Different passages of MCF-7 cell (MCF-7 p19, MCF-7 p72) and their variants (LCC-1, LCC-2, LCC-9, LY-2 and R27) all showed the feature of significant amplification of AIB1 gene. The higher amplification of AIB1 in LCC-1, LCC-9, LY-2 than in their original MCF-7 cells suggest that the gaining of estrogen independence may result in the higher level of amplified AIB1 gene. These data indicate that amplification of AIB1 gene is regulated by hormonal status. Sequencing analysis of poly Q encoding region of AIB1 gene showed that 26% of breast cancer cell lines had 3 or more poly Q repeat encoding sequence patterns. Among them, MD-MB-157, MD-MB-435 and BT-483 had 6, 4, 4 distinct poly Q sequence patterns respectively. LCC-6, the variant of MD-MB-435, even had 7 distinct poly Q sequence patterns. Although all MCF-7 cells and their variants had the same predominant poly Q encoding sequence pattern of (CAG)₃CAA(CAG)₉(CAACAG)₃(CAACAGCAG)₂CAA of the parental cell line, small percentage of altered poly Q sequences were found in all MCF-7 passages and variants. These data suggest somatic instability of AIB1 gene. In conclusion, the poly Q encoding DNA sequence of AIB1 gene is somatically unstable. Its amplification partially depends on the presence of ER and level of amplification may be affected by the estrogenicity of the cellular environment.

Human methylthioadenosine phosphorylase, a gene frequently co-deleted with p16, acts as a tumor suppressor gene. *W.D. Kruger¹, S.A. Christopher¹, P. Diegelman², C.W. Porter².* 1) Population Sci, Fox Chase Cancer Ctr, Philadelphia, PA; 2) Pharmacology and Therapeutics Department, Roswell Park Cancer Institute, Buffalo, NY.

The human MTAP gene is located on 9p21 and is frequently homozygously deleted, along with p16/ARF, in a wide variety of human tumors and human tumor derived cell lines. MTAPs function is to salvage methylthioadenosine (MTA) which is produced as a byproduct of polyamine metabolism. We have reintroduced MTAP into MCF-7 breast adenocarcinoma cells and have examined its effect on the tumorigenic properties of these cells. MTAP expression does not affect the growth rate of cells in standard tissue culture conditions, but severely inhibits their ability to form colonies in soft-agar or collagen. In addition, MTAP expressing cells are suppressed for tumor formation when implanted into SCID mice. This suppression of anchorage-independent growth appears to be due to MTAPs enzymatic activity, as a protein with a missense mutation in the active site does not exhibit this phenotype. MTAP expression causes a significant decrease in intracellular polyamine levels and alters the ratio of putrescine to total polyamines. Consistent with this observation, the polyamine biosynthesis inhibitor α -difluoromethylornithine (DFMO) inhibits the ability of MTAP deficient cells to form colonies in soft-agar, while addition of the polyamine putrescine stimulates colony formation in MTAP expressing cells. These results indicate that MTAP has tumor suppressor activity and suggest that its effects may be mediated by altering intracellular polyamine pools.

Epigenetic control of genomic stability in an endometrial cancer-derived cell line. *N. Joshi, S.G. Grant.*
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It is widely assumed that genomic instability is an early development in carcinogenesis. In a survey of breast and endometrial cancer-derived cell lines, however, we found that baseline frequencies of mutation at the X-linked HPRT locus varied by over three orders of magnitude, from approximately normal frequencies of 10^{-6} to frequencies approaching 10^{-3} . Cell lines with mutation frequencies at the upper end of this range could not be induced with the alkylating agent ethylmethane sulfonate. The same broad range of mutation frequencies could be observed within sublines of the breast cancer cell line MCF7 and the endometrial cancer cell line Ishikawa. To determine whether the genomic stability phenotype of the Ishikawa-ECCA subline, with a mutation frequency of 2.6×10^{-6} , was subject to culture conditions, we grew these cells under conditions of restricted serum (1 and 2% vs. 5, 10 and 15%), which are selective for transformed cells. After one month of growth under these selective conditions the mutation frequency of these cells rose dramatically to 10^{-3} , similar to the frequency observed in the Ishikawa-UNC subline, 3.6×10^{-3} . Similarly, cells grown for 3 and 7 months under these conditions also exhibited mutation frequencies in the range of 10^{-3} or more. Perhaps more significantly, similar increases in uninduced mutation frequency were observed when the Ishikawa-ECCA cells were incubated for only one week in low serum medium, or if they were only selected in this medium. Finally, if the cells grown in 1% serum for one month were returned to 15% serum for one month, they reacquired their original genetic stability. It should be noted that growth in low serum resulted in long doubling times and low cloning efficiencies, even after months of culture under these conditions. One interpretation of these results is that genomic instability arose during the generation of the tumor this cell line was derived from, but that the full tumorigenic phenotype is not expressed under normal, i.e. high serum, culture conditions. These results have important implications for in vitro studies of the fundamental characteristics of the transformed phenotype.

The E2F binding partner DP-1 and its role in cancer progression and differentiation. *M.T. Tilli^{1,4}, M.S. Frech⁴, S.L. Hudgins¹, J-P. Renou³, I. Heletz¹, K.S. Hruska^{1,2}, L. Hennighausen³, J.A. Flaws², P.A. Furth^{1,4}.* 1) Graduate Program in Human Genetics, University of Maryland, Baltimore, Baltimore, MD; 2) Department of Epidemiology and Preventive Medicine, University of Maryland, Baltimore, Baltimore, MD; 3) Laboratory of Genetics and Physiology, NIDDK, NIH, Bethesda, MD; 4) Lombardi Cancer Center, Department of Oncology, Georgetown University, Washington, DC.

The aim of this study was to examine genetic events controlling cancer progression and regression using conditional transgenic mouse models. The T-Antigen (TAg) conditional carcinogenesis model with reversible (4 mo. TAg expression) and irreversible (7 mo.) stages of dysplasia in the salivary gland (SG) was utilized. Differential expression of genes needed for reversal was examined by probing a mouse oncochip cDNA microarray (NCI) of 2600 cancer pathway genes with SG RNA from 4 mo. and 7 mo. old tTA/TAg mice. Two-fold increased expression at 7 mo. was seen in 2 cell cycle regulatory genes, including DP-1, dimerization partner essential for activity of E2F transcription factor family, suggesting a role in reversal. DP-1 was localized to TAg-targeted SG cells in 4 and 7 mo. mice and in SG tumors. Western blot analysis comparing 4 mo. tTA/TAg mice treated with doxycycline revealed 3 closely migrating forms of DP-1. The forms of DP-1 may be due to differences in phosphorylation, with the highest form involved in cellular differentiation, the middle form in cell cycle progression, and the lowest form in cellular quiescence. PPAR γ induces phosphorylation of DP-1, leading to decreased DNA-binding activity of the E2F-DP complex due to a decrease in the ubiquitous serine-threonine phosphatase, PP2A. Treatment with PP2A altered the mobility of the highest form of DP-1, suggesting that DP-1 is differentially phosphorylated during tumorigenesis in this model. ERa's role in cancer was examined by introducing a novel mouse model with conditional dominant gain of ERa (tTA/ERa) into the TAg model. Three tTA/TAg/ERa females, but no tTA/TAg females, developed mammary gland (MG) tumors, suggesting that overexpression of ERa in this model may contribute to MG tumor development.

Somatic alterations of the mitochondrial genome in human hepatocellular carcinomas. *D.J. Tan¹, L. Agress¹, L.L. Liu¹, R.K. Bai¹, J. Chang², L.J. Wong¹.* 1) Institute for Molecular and Human Genetics, Georgetown University Medical Center, Washington, DC; 2) Changhua Christian Hospital, Taipei, Taiwan.

Mitochondria are not only the sites where the reactive oxygen species are generated but also the easy target for oxidative DNA damage. Recent reports have supported the concept that somatic mitochondrial DNA (mtDNA) alteration is a general phenomenon of cancer. In order to investigate the potential role of somatic mtDNA mutations in tumorigenesis, the occurrence of mutations in mtDNA of hepatocellular carcinomas was studied. Somatic mtDNA mutations of the entire mitochondrial genome were screened in 20 pairs of hepatic tumor and surrounding tissues using 32 overlapping PCR primer and temporal temperature gradient gel electrophoresis (TTGE). DNA fragments showing different TTGE banding patterns between normal and tumor mtDNA were followed by direct DNA sequencing to identify the mtDNA mutations. Our results showed that 12 out of 20 (60%) tumors had somatic mtDNA mutations. Eleven of them (55%) had mutations in the hypervariable D loop region, and One had mutation in mRNA. Nine tumors had one mutation, each of the remaining had multiple somatic mutations with a total of 27 mutations. All mutations, except one, 26/27(94.3%) were in the hypervariable D loop region. Among them, 21 were in H-strand origin area (80.7%). Eight were involved in np303-315 poly C region. Quantitative PCR analysis showed that the amount of mitochondrial DNA per cell in 7 tumors was below 50% of the mean. One tumor with a stretch of 12->13 Cs in np303-315 had a 2.7 fold elevation in mtDNA content. Twenty-two mtDNA alterations were at the homoplasmic state in tumor tissue, 5 of them were heteroplasmic state in the surrounding normal tissue. The remaining 5 mutations were heteroplasmic state in tumor. Our study suggests that there is a significantly higher incidence of D loop mtDNA mutations in hepatocellular carcinomas (94.3%) compared to breast cancer (81.5%) and oral cancer (40%). The high instability of poly C tract at np303-309 might play a role in tumorigenesis.

DNA repair gene SNPs as modifiers of BRCA1 and BRCA2 phenotypic expression. *G. Chenevix-Trench*¹, *A. Spurdle*(*kConFab*)¹, *X. Chen*¹, *J. Hopper* (*ABCFS*)², *C. Apicella* (*AJS*)², *B. Newman*³, *M. Cook*⁴, *S. Peock*⁴, *D. Easton* (*EMBRACE*)⁴. 1) Queensland Institute of Medical Research, Brisbane, Australia; 2) University of Melbourne; 3) School of Public Health, Queensland University of Technology, Brisbane; 4) CRC Genetic Epidemiology Unit, University of Cambridge, UK.

We are assessing the role of DNA repair gene variants as modifiers of BRCA1 or BRCA2 mutations, and as low-penetrance breast cancer genes. 552 female BRCA1 and BRCA2 mutation carriers from 337 families, collected through Australian (kConFab, AJBCS, ABCFS) and British sources (EMBRACE), have been screened for the Breakage Excision Repair APE Asp148Glu and XRCC1 Arg399Gln SNPs. Modification of disease presentation was assessed by analysis of shifts in genotype distribution with respect to affected status, and by comparison of age at onset of breast cancer for different genotypes. There was no evidence that the APE 148Glu variant modified disease presentation in the BRCA1 or BRCA2 carriers, for crude analyses or after adjustment for sample source, ethnicity and oophorectomy. However, there was evidence that the XRCC1 Gln allele modified disease presentation in BRCA1 carriers, with a lower frequency of heterozygotes and Gln homozygotes in affected vs unaffected carriers ($P=0.04$). Affected BRCA1 carriers with at least one Gln allele had a marginally older age at onset of breast cancer ($P=0.04$). The XRCC1 variant did not modify disease presentation amongst BRCA2 carriers ($P>0.6$). A population-based case-control-family sample of Australian women, over-sampled for early-onset disease, has been screened for the same BER variants. Results from 1455 cases and 793 controls suggest that the APE Asp148Glu is not associated with breast cancer risk, with ORs of 1.00 (0.81-1.25) and 1.02 (0.80-1.29) for the heterozygote and homozygote Glu genotypes, respectively. The XRCC1 Arg399Gln substitution also occurs at similar frequencies in cases and controls with ORs of 0.94 (0.78-1.14) and 0.98 (0.75-1.30) respectively, for the heterozygote and homozygote Gln genotypes. Analyses with stratification by exposure to environmental risk factors are pending, as are analyses of an additional 95 BRCA1 and BRCA2 carriers.

An investigation of the relationship between DNA repair genotype and histopathological subtype in breast cancer. A. Cox¹, S. Rafii¹, G. Xinarianos¹, I. Azmy², M. Reed², T. Stephenson³, M. Meuth¹. 1) Institute for Cancer Studies, University of Sheffield, UK; 2) Section of Surgical and Anaesthetic Sciences, University of Sheffield, UK; 3) Department of Histopathology, Royal Hallamshire Hospital, Sheffield, UK.

There is considerable evidence to suggest that defects in DNA damage repair, in particular the homologous recombination repair (HRR) pathway of DNA double strand break repair, contribute to breast cancer susceptibility. Recent studies from our own laboratory and others have shown that common polymorphisms in genes involved in HRR, including the *XRCC2* and *XRCC3* genes, have modest effects on sporadic breast cancer risk. To clarify the role of *XRCC2* and *XRCC3* polymorphisms in breast cancer in more detail, we investigated whether they were associated with particular histopathological subtypes. Women with histologically confirmed breast cancer were recruited from surgical out-patient clinics in Sheffield, UK, and detailed demographic, clinical, and histopathological data and a blood sample were obtained from each patient. Genotyping of the *XRCC2 R188H* and *XRCC3 T241M* polymorphisms was performed by 5' nuclease PCR (Taqman™) and PCR-SSCP. Histopathological data were available on 387 tumours; 376 of these were invasive lesions, the remainder were ductal carcinoma in situ. The majority (272, 72.3%) of the invasive tumours were of the ductal NOS (not otherwise specified) type, 40 (10.6%) were lobular, and 64 (17.0%) were ductal special types. The rate of carriage of the *XRCC2 188H* allele was raised in the lobular tumours compared to the ductal NOS tumours (27.5% vs 15.1%, $p=0.05$), with a similar trend for the rate of carriage of the *XRCC3 241M* allele (72.5% vs 58.5%, $p=0.09$). The allele carriage rates in the ductal sub-types were similar to those in the ductal NOS tumours (18.75% and 62.5% respectively). There was no association between either of the polymorphisms and tumour grade or oestrogen receptor status. Whilst these studies require confirmation, they raise the possibility that polymorphisms of the HRR pathway may be relevant to particular breast tumour subtypes.

African-Americans and Caucasians differ in polymorphisms of Glutathione S-transferases and Estrogen Receptor b genes correlated with prostate cancer. *K. Muralidharan, E. David.* Department of Pediatrics, Division of Genetics, Emory University School of Medicine, Atlanta, GA.

The incidence of prostate cancer is the highest among African-Americans. The reason for this increased incidence is not known. Differences in certain polymorphic genes involved in carcinogen detoxification in the prostate or in prostate development and differentiation could contribute to this difference. Polymorphisms in Glutathione S-transferase genes (GSTM1, GSTT1, and GSTP1 isotypes) have been correlated with prostate cancer susceptibility. We compared the frequency of GST variants in a group of African-Americans and Caucasians. Our results revealed that the genotype frequencies are statistically different between these two races, particularly for GSTM1 and GSTP1 variants [$p < 0.001$]. Estrogen receptor [ER] plays a critical role in prostate cancer apart. We studied the CA repeat polymorphism in ER b gene in the African-Americans and Caucasians. These two groups differ significantly in the range and distribution of CA repeats. African-Americans have an extended range of CA repeats compared to Caucasians. The allele with 23 repeats was the most common in African-Americans, while the allele of 27 repeats was most common in Caucasians. We conclude from our preliminary results that African-Americans and Caucasians differ significantly in these polymorphisms that have been correlated with prostate cancer.

Ornithine decarboxylase allele frequency distribution in Caucasians and African Americans. *T.G. O'Brien¹, A. Parlanti², Y. Guo¹, D. Boorman¹, K. Visvanathan³, K. Helzlsouer³, D. Watkins-Bruner².* 1) Lankenau Inst for Med Research, Wynnewood, PA; 2) Fox Chase Cancer Center, Philadelphia, PA; 3) Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.

Differences in prostate cancer incidence and mortality between North American Caucasian and African American men are unexplained by known risk factors. Allelic variation at common, low penetrance cancer susceptibility/resistance loci may contribute to these differences in disease risk. A functional single nucleotide polymorphism (SNP) in the human ornithine decarboxylase (ODC) gene has been described that defines 2 allelic variants, termed A and G. Inheritance of one or two copies of the ODC A allele is associated with increased risk of prostate cancer in North American Caucasians who are cigarette smokers and in those men who carry an androgen receptor (AR) gene with less than or equal to 22 CAG repeats. We hypothesized that the frequency of the ODC A allele may be increased among African American compared to North American Caucasian men and that the mean number of AR CAG repeats may be decreased. In order to evaluate this, 150 African American men in the Prostate Cancer Risk Assessment Program (PRAP) at Fox Chase Cancer Center were genotyped for both AR CAG repeats and ODC. The results were then compared to those obtained from a population-based study from Washington County, MD, of 319 Caucasian men. The distribution of ODC genotypes (AA, AG, and GG) in African-Americans was significantly different ($P=0.0005$, Chi-square test) than the ODC genotype distribution in Caucasians: 13.3% of African-Americans had the AA genotype vs. 6.6% of Caucasians and 42% of African-Americans were AG vs. 35.7% of Caucasians. Thus, the allele frequency of the putative high-risk A allele in African-Americans (0.343) was 40% higher than in Caucasians (0.247). Consistent with other studies, the mean number of AR CAG repeats was 19.8 in African Americans compared to 22.0 in Caucasians. It is possible that the higher frequencies of risk alleles for both the ODC and AR genes in African Americans compared to Caucasians may account for some of the difference in prostate cancer risk between these two ethnic groups.

RNASEL variation does not account for the increased risk of prostate cancer in the Afro-Caribbean population of Tobago. *P.R. Shea¹, C.S. Ishwad¹, R.E. Ferrell¹, A.L. Patrick³, L.H. Kuller², C.H. Bunker².* 1) Human Genetics, Univ Pittsburgh, Pittsburgh, PA; 2) Epidemiology, Univ Pittsburgh, Pittsburgh, PA; 3) Tobago Regional Health Authority, Scarborough, Tobago, Tobago and Trinidad.

Recently, the Ribonuclease-L gene was implicated as a positional candidate gene for prostate cancer (PCa) susceptibility in the HPC1 region of chromosome 1q24-25. Two polymorphic missense variants, Arg462Gln and Glu541Asp, and a Glu265X truncation variant were reported to confer an increased risk of hereditary PCa. Afro-Caribbean males from Tobago have an elevated screening-detected prevalence of PCa (135/1000 in males > 49 years of age). However, only a small number of patients of African ancestry were included in previous studies of RNASEL. Because a number of African-American families were included in the original linkage study that localized HPC1, we tested the hypothesis that variation in RNASEL influences risk of prostate cancer in the population of African ancestry in Tobago. We genotyped 104 cases and 228 controls for the Arg462Gln and Glu541Asp variants identified in RNASEL. The 462Gln allele was found to be much less common in the Tobago population (12.4% compared to 63%) than in the previously reported European/European-American population. The 265X variant was not observed in 48 Tobago prostate cancer cases screened by direct sequencing. The Glu541Asp polymorphism was not significantly associated with risk of PCa in the Tobago population. Alternatively, homozygotes for the 462Gln allele were found to have an increased risk of borderline significance ($p=0.068$). Because of the low frequency of the 462Gln allele in Tobago, neither homozygosity for this allele nor variation at the Glu541Asp site can account for the increased risk of prostate cancer observed in this population.

Profiling SMAD4-mediated transcriptional regulation by TGF-beta on a global scale using high density microarrays. *P.J. Collins¹, T. Cheung², T.B. Doan¹, K.W. Shannon¹, X. Liu².* 1) Agilent Technologies, 3500 Deer Creek Road, 25U, Palo Alto, CA 94304; 2) Department of Chemistry and Biochemistry, UCB215, University of Colorado-Boulder, Boulder, CO 80503.

Transforming growth factor-beta is a multifunctional growth factor whose best-known function is to inhibit cell growth and suppress tumor formation. Loss of TGF-beta growth inhibition is one of the most common cellular events in the pathogenesis of human breast, pancreatic and colon cancers. TGF-beta signals through a heteromeric signaling complex consisting of Smad2, 3 and 4. Disruption of the Smad signaling complex often leads to tumor formation. We have used both 60-mer oligonucleotide and cDNA microarrays to investigate the consequences of Smad4 inactivation to the TGF-beta controlled transcription program in tumor cells. These high density DNA microarrays, generated using Agilent's SurePrint inkjet technology, were used to profile global transcriptional regulation in breast, colon and pancreatic Smad4-null tumor cell lines in response to TGF-beta. Data from both microarray types showed a high degree of correlation in demonstrating that TGF-beta induces transcriptional activation and repression of genes involved in signal transduction, cell adhesion and transcriptional regulation across the range of cell lines tested. Comparison with expression profiles from Smad4-positive cell lines indicates that inactivation of Smad4 does alter transcriptional programs of TGF-beta signaling but it is not absolutely required for TGF-beta signaling from cell surface to nucleus. The composition of the Smad signaling complex may control the specificity of TGF-beta signaling to control expression.

Transforming Growth Factor-beta Receptor type III expression levels are downregulated in cancerous tissues.
C.M. Stevens, M. Dressman, C.D. Wolfgang, C. Lavedan. Pharmacogenetics, Novartis Pharmaceuticals Corp, Gaithersburg, MD.

Transforming growth factor- beta (TGF-B), is a cytokine involved in many different cell growth and repair functions including cell differentiation, adhesion, and cell cycle progression. To date, three forms of the TGFB receptor have been identified. Even though TGFB binds to the type III receptor (TGFBR3), this receptor lacks a recognizable signaling domain and has no defined role in TGFB signaling. To better understand the expression of this receptor in different human tissue types, which may provide insight into its function, we examined the expression level of TGFBR3 in 50 different tissues using RNA expression profiling. While most non-cancerous tissues express TGFBR3 at some level, the expression of TGFBR3 is downregulated in all cancerous tissues with the exception of stomach. Reproductive tissues such as breast, cervix, ovary, and testes showed the most significant differences between normal and cancerous tissues. On average, the cancerous tissues showed 11.86 fold lower levels of TGFBR3 expression compared to their normal matched tissue. This downregulation in expression of TGFBR3 indicates that the gene may have tumor suppressor activities.

Androgen receptor (CAG)_n and (GGC)_n polymorphisms and breast cancer risk in a population-based case control study of young women. *N.M. Suter¹, K.E. Malone², J.R. Daling², D.R. Doody², E.A. Ostrander¹.* 1) Human Biology, Fred Hutchinson CRC, Seattle, WA; 2) Public Health Sciences, Fred Hutchinson CRC, Seattle, WA.

The androgen receptor (AR) is involved in the regulation of hormone responsive genes, and as such, has been suggested as a possible candidate gene for breast cancer susceptibility. The AR gene contains two polymorphic trinucleotide repeats within exon 1: (CAG)_n and (GGC)_n, encoding for polyglutamine and polyglycine tracts respectively. Few studies have assessed both repeats with respect to breast cancer risk in the general population, and none have done so in young women with breast cancer. Women diagnosed with breast cancer before age 45 and age-matched controls, all participants in a population-based case-control study of breast cancer before were therefore assessed for length variation in the (CAG)_n and (GGC)_n AR repeats.

Results were generated from 524 cases and 461 controls. Repeat lengths were analyzed as categorical variables and adjusted for age and reference year. Women with a cumulative (CAG)_n repeat size of 43 showed a modest increased risk for breast cancer (OR =1.3, 95% CI 1.0-1.7). Women with a (GGC)_n Long (L) allele and those with a 33 cumulative repeat size had a significantly decreased risk of breast cancer (OR=0.7, 95% CI 0.5-0.9). Among women homozygous for the (CAG)_n Short (S) allele, and among women with any (GGC)_n L allele, there was an increased risk of breast cancer in relation to ever use of oral contraceptives (OR=1.9, 95% CI 1.0-3.6 and OR = 1.7, 95% CI 0.9-3.5, respectively). Risk did not increase further with duration or recency of last use. Our analyses are the first to assess breast cancer risk in young women from the general population in relation to both (CAG)_n and (GGC)_n repeats in the AR gene, and suggest a reduced risk for those who possess (GGC)_n repeat lengths of 17 or greater. In addition, our results suggest AR repeat length may be partly responsible for the increased risk for breast cancer in women who use oral contraceptives. Further work is needed to confirm these findings.

Hereditary hemochromatosis in a large mexican family. *L.R. Cornejo^{1,2}, H. Romero¹, L. Oliver¹, G. Nava¹.* 1) Medicine Academic Area, Autonomous Hidalgo State Univ, Pachuca de Soto, Hidalgo, Mexico; 2) Childrens Hospital of Mexico Federico Gomez, Mexico City, Mexico.

Introduction. Hereditary hemochromatosis (HH) is one of the most common autosomal recessive disease. Its frequency has been estimated between 1/200 to 1/400 in Caucasian population. HH has been classified in three types regarding its clinical presentation. Its full phenotype keeps several questions because it is vary variable. To approach patients with HH include, (1) clinical data in liver, heart, pancreas, endocrine organs, skin and joints; (2) lab test such as serum iron, serum ferritin and transferrin saturation; and, (3) molecular biology studies for HFE and TFR2 genes. Geographic localization of Zimapan, Hidalgo Mexico has been highly associated with arsenic residues pollution. Objective. To present a large pedigree with HH where there are 36 relatives under risk to develop the disease. Material. Thirty-six people who are first and second degree relatives of an already dead 18 years old male proband. Methods. Clinical diagnosis and lab test in proband, as well as, clinical evaluation for his available relatives pictured from a three generation pedigree. Results. An eighteen years old male was studied, he presented the next clinical characteristics: cardiomyopathy, diabetes mellitus, hypogonadism, skin pigmentation, arthritis and hepatic cancer; and, lab test alterations. His pedigree shows a brother who dead previously because of the same liver disease. One of his sisters is developing hepatic alterations since 3 months ago. Three of his first degree relatives and 6 second degree relatives have skin pigmentation. Discussion. HH was thought as diagnosis because clinical aspects. It is show that develop of the disease is variable for male and female. There is a strong recommendation to establish a pathway to preserve an hepatic integrity, after enzyme restriction assay and sequencing in the relatives under risk. Conclusions. (a) A first large mexican family is presented; (b) to outline the major clinical date as hepatic cancer in young people; and, (3) it remains molecular biology test as support the correlation with arsenic pollution background where the family is living.

A variant of Tietz syndrome caused by a mutation in the basic domain of the MITF gene. *K.O. Welch¹, S.D. Smith², D. Hoover², K.S. Arnos¹, P.M. Kelley³, A. Pandya⁴, W.E. Nance⁴.* 1) Biology Dept, Gallaudet Univ, Washington, DC; 2) Ctr for Human Molecular Genetics, Univ of Nebraska Medical Ctr, Omaha, NE; 3) Ctr for Hereditary Communication Disorders, Boys Town National Research Hospital, Omaha, NE; 4) Dept of Human Genetics, Virginia Commonwealth Univ, Richmond, VA.

Tietz syndrome is a dominant form of profound sensorineural hearing loss (SNHL) with generalized hypopigmentation of the skin and hair, blue irides with hypopigmented fundi, and complete penetrance. The only family reported with these features has a missense mutation (Asp210Lys) in the basic domain of the MITF gene. Another missense mutation (Arg217del) in the basic domain was reported in a deaf mother and son. The mother had red hair with premature greying, fair skin and freckles, and blue eyes with hypopigmented fundi. Various mutations in other domains of MITF cause Waardenburg syndrome, type II (WS2), a dominant condition with SNHL of varying degrees that may be bilateral or unilateral, heterochromia irides, white forelock, patchy hypopigmentation of the skin, and variable expression. Our family has 28 members with features of both Tietz syndrome and WS2. All have blue eyes and generalized hypopigmentation of the skin and hair. Some have a white forelock and premature greying. Many have small pupils and photophobia; none have nystagmus. Several individuals have constipation, some severe. Fourteen are hearing. Fourteen have varying degrees of SNHL, including two with unilateral deafness. Sequencing of MITF revealed a T to G substitution in exon 6 in the basic domain, resulting in the missense mutation Ile212Met. This mutation was found in all 6 affected family members who were tested but not in the one unaffected family member tested nor in 96 controls. The mutation is in the same amino acid as the mouse Mi mutation that causes a dilute (as opposed to spotted) phenotype. Other mutations in this region are associated with diffuse hypopigmentation in mice and Tietz syndrome in humans. Spotted/WS2 phenotypes are associated with mutations in other regions or truncation mutations. While this family shows phenotypic overlap between the two syndromes, the generalized hypopigmentation and blue eyes are requisite features.

Characterization of osteogenesis imperfecta diagnosed in adulthood. *L.J. Kessler¹, M. Pepin², P.H. Byers², J. Farmer¹, R.E. Pyeritz¹.* 1) Medical Genetics, Univ Pennsylvania, Philadelphia, PA; 2) Dept Pathology, Univ Washington, Seattle.

Osteogenesis imperfecta (OI) syndromes share osteopenia due to a deficiency of the osteoid component of bone and osteoporosis, but are diverse in severity, prognosis and cause. Most OI is due to mutations in either COL1A1 or COL1A2. In the past 2 years, we established the diagnosis of OI in 5 adult patients (pts). Here we review their clinical, biochemical and genetic characteristics. All 5 pts were women (ages 34, 42, 52, 57 & 58 yr); 3 were postmenopausal. As children, 1 patient was diagnosed with rickets and 1 had probably been diagnosed with OI, but pediatric records were never transferred to adult physicians. Each was being evaluated for different medical problems, and was referred to genetics because of severe osteoporosis. 3 pts had no relatives with unusual fractures or osteoporosis and 1 had not had children. The fracture history was diverse, including 1 pt with 0, 1 with 2 after trauma as an adult, 1 with 3 as a child and 0 as an adult, 1 with 2 as a child and more than 10 as an adult, and 1 with many as a child and 0 as an adult. All were short-statured and had blue sclerae. 3 had dentinogenesis imperfecta (DI) and 1 who was edentulous probably did. The 4 who had DEXA scans had osteopenia (T scores -1.6 to -3.9). Collagen synthesis by dermal fibroblasts was assessed in 2 and was pending in 2. One pt synthesized normal type I collagen. One pt with an extensive pedigree of OI had a normal type I to type III collagen ratio; biochemical studies on the other pts and mutational analyses are pending. In conclusion, OI may present in adulthood, both in pts never diagnosed and in some without precise knowledge of past medical history. Women are particularly prone to be diagnosed because of general concerns about bone density, especially post-menopause. OI should be suspected in adults who present with moderate osteopenia, a history of fractures at any age or occult fractures, blue sclerae or DI. The role of collagen studies will vary among pts, but is especially useful in pts of child-bearing age to confirm the diagnosis and to offer prenatal and obstetrical counseling.

A new proximal mutation of the receptor tyrosine kinase gene *ROR2* associated with a severe form of brachydactyly type B. C. Carves-de La Rochebr^{1, 2}, V. Drouin-Garraud¹, N. Drouot¹, C. Martin¹, B. Bachy³, T. Frebourg^{1, 2}. 1) Department of Genetics, CHU de Rouen, 76031 Rouen, France; 2) INSERM EMI 9906, Faculty of Medicine, 76183 Rouen, France; 3) Department of Pediatric Surgery, CHU de Rouen, 76031 Rouen, France.

Heterozygous mutations of the orphan receptor tyrosine kinase *ROR2* gene are responsible for brachydactyly type B (BDB), an autosomal disorder characterized by terminal deficiency of fingers and toes. In a French family, we identified a new frameshift mutation, 1394delT, localized within the proximal mutations cluster, and responsible for a severe form of BDB. The index cases mother exhibited a supernumerary lumbar vertebra, which might be part of the BDB phenotype associated with *ROR2* mutations. The association of a mutation localized within the proximal region and a severe phenotype moderates the genotype/phenotype correlation previously suggested in BDB. This report suggests that the severity of the BDB phenotype depends more on modifying factors than on the location of the mutation itself.

Clinical Homogeneity and Genetic Heterogeneity in Weill-Marchesani Syndrome. *L. Faivre¹, MK. Wirtz², R.J. Gorlin³, A. Megarbane⁴, H. Dolffus⁵, S. Lyonnet¹, Y. Alembik⁵, A. Alswaid⁶, M. Le Merrer¹, A. Munnich¹, V. Cormier-Daire¹.* 1) Dept Genetics, Hopital Necker Enfants Malades, Paris, France; 2) Department of Ophthalmology, Oregon Health Sciences University, Portland, Oregon, USA; 3) Department of Oral Science, University of Minnesota Health Sciences Center, Minneapolis, Minnesota, USA; 4) Medical Genetics, Saint Joseph University, Beirut, Lebanon; 5) Medical Genetics, Hopital de Hautepierre, Strasbourg, France; 6) Clinical Genetics, Riyadh Armed Forces Hospital, Riyadh, Saudi Arabi.

Weill-Marchesani syndrome (WMS) is a rare disease characterized by short stature, brachydactyly, joint stiffness, and characteristic eye abnormalities including microspherophakia, ectopia of lens, severe myopia and glaucoma. Both autosomal recessive (AR) and autosomal dominant (AD) modes of inheritance have been described in association with WMS and one locus for AR WMS has recently been mapped to chromosome 19p13.3-p13.2. Here, we report on the exclusion of linkage to chromosome 19p13.3-p13.2 in a large AD WMS family using twelve microsatellite polymorphic markers (distant from 1.5 cM) encompassing the locus. In order to answer the question of whether or not genetic heterogeneity could be related to a clinical heterogeneity, we reviewed 128 WMS patients from the literature (including 57 AR, 50 AD and 21 sporadic cases), with a particular attention for clinical features. Statistical analyses using Fischer exact test were used to compare the proportions of 12 clinical parameters between AR and AD patients. There was no significant difference between both groups for myopia, glaucoma, cataract, short stature, brachydactyly, thick skin, muscular build and mental retardation. Significant results were found for microspherophakia (94% in AR, 74% in AD, Fischer 0.007), ectopia lentis (64% in AR, 84% in AD, Fischer 0.0063), joint limitations (49% in AR, 77% in AD, Fischer 0.010) and cardiac anomalies (39% in AR, 13% in AD, Fischer 0.004). Nevertheless, we failed to distinguish AR from AD inheritance in individual cases. These results support the clinical homogeneity but the genetic heterogeneity of WMS.

Quantitative ultrasound at the phalanges in a cohort of monozygotic twins: evaluation of affinities and environmental influences. *G. Guglielmi¹, F. De Terlizzi², I. Torrente³, R. Mingarelli³, M. Cammisa¹, S.B. Angeletti⁴, B. Dallapiccola⁵.* 1) Department of Radiology, IRCCS - CSS, S.Giov. Rotondo, Italy; 2) Laboratory of Clinical Biophysics IGEA, Carpi, Italy; 3) Mendel Institute, Rome, ITALY; 4) New York Accademy of Science, New York, NJ; 5) IRCCS-CSS, S.Giov.Rotondo and Mendel Institute, Rome, Italy.

In this study we have investigated the ultrasonographic characterisation of a cohort of 79 couples of Italian monozygotic twins: 56 couples of female twins and 23 couples of male twins. The 158 subjects were on an age range between 7 and 60 years and have been measured for ultrasonic evaluation of the bone tissue at level of the distal metaphysis of the first phalanx of the last four fingers of the non dominant hand by DBM Sonic Bone Profiler (IGEA, Italy). Data of Amplitude Dependent Speed of Sound (AD-SoS) and Bone Transmission Time (BTT) have been collected; in particular BTT was of great interest because of its complete independence on soft tissue thickness around the phalanx. Data have been analysed all together and separately for males and females couples. Extremely high correlation coefficients were found for AD-SOS and BTT when comparing all couples of twins. R values were: 0.91 for AD-SoS (0.89 for females only, 0.95 for males only) and 0.95 for BTT (0.92 for females only, 0.95 for males only). All correlation coefficients were statistically significant ($p < 0.0001$). Finally we investigated the influence of age in the progressive differentiation between twins, observed by ultrasound parameters. We classified twins into 5 groups according to their age and we calculated the mean difference between couples of twins for AD-SoS and BTT values in each group. For female groups ANOVA results show a statistical significance ($p < 0.05$) in the association between increasing differences in AD-SoS and BTT between couples of twins with increasing age. This result was not observed on the male group of twins, probably due to the low number of subjects. In conclusion these results support the evidence of an environmental influence on bone status in our studied group of MZ twins.

Genital anomalies in a boy with sporadic brachydactyly, type A2. *G.A. Bellus¹, J. Anderson²*. 1) Depts Dermatology, Pediatrics and Human Medical Genetics Program, Univ Colorado Health Sci Ctr, Denver, CO; 2) Genetic Services, The Children's Hospital, Denver, CO.

Brachydactyly, type A2 (BDA2) is an extremely rare genetic disorder characterized by a triangular middle phalanx in the index finger, often resulting in striking radial deviation, and mild fifth finger clinodactyly due to abnormal deviation of the distal phalanges. In the feet, the halluces are often short, broad and laterally deviated and the remaining toes, especially the second toes, have hypoplastic middle phalanges, resulting in medial deviation of their distal phalanges. There are very few reports of BDA2 in the literature and no mention of other associated findings. Our patient was the product of a G1 P0-1 mother. Triple screen revealed elevated AFP leading to fetal u/s and genetic amniocentesis. The ultrasound was suspicious for distal limb anomalies and the karyotype was 46, XY. He was delivered at 38 weeks by vaginal delivery and presented with classic features of BDA2 as well as severe hypospadias with a bifid scrotum and a perineal opening. Both parents have normal limbs and genitalia and rest of the family history was unremarkable. Brachydactyly and genital anomalies are observed in Hand-Foot-Genital syndrome (HFGS) which, is due to mutations in the Hox A13 gene. However in HFGS, it's always the first digits that are most obviously involved, resulting in short proximally-placed thumbs, hypoplastic thenar eminences and short medially-deviated halluces, with small pointed first distal phalanges and short first metacarpals/metatarsals. Our patient has normal thumbs, and his toes are broad and laterally deviated. In HFGS, there is often also hypoplasia of the middle phalanges, especially in the little and index fingers, and in toes 2 to 5, and this can result in fifth finger clinodactyly and ulnar deviation of the index fingers - but not the striking radial deviation demonstrated by our patient. The occurrence of severe hypospadias and BDA2 in this patient may be coincidental but could also represent a new association. The general similarities between HFGS and the findings in our patient suggest that mutations in another Hox gene may cause BDA2.

Mutation detection in *NOGGIN* in individuals with multiple synostosis syndrome. *K.G. Dawson, E. Sebald, J.M. Graham, Jr., D. Krakow.* Department of Pediatrics, Division of Medical Genetics, Cedars-Sinai Medical Center, UCLA School of Medicine, Los Angeles, CA.

Multiple synostosis syndrome (SYNS1; OMIM 186500) is an autosomal dominant disorder characterized by early, progressive joint fusions, characteristic facies, and deafness. The disorder was mapped to chromosome 17q21-22, the location of the disease gene for proximal symphalangism (SYM1; OMIM 185800). Subsequently, heterozygous mutations in *NOGGIN* (*NOG*) were identified in the allelic disorders. Noggin has been shown to be a bone morphogenetic protein antagonist, and mice homozygous for *Nog*-null alleles have several skeletal developmental defects, including a lack of normal articulating joints. To date, eleven *NOG* mutations have been published, all of which were within the single coding exon. We identified eight individuals with the clinical findings characteristic of multiple synostosis syndrome. Seven of these occurred as sporadic cases. To identify novel multiple synostosis syndrome mutations in the *NOG* gene we sequenced the entire coding region of *NOG* in both 5' to 3' and 3' to 5' directions. We were able to demonstrate a previously published mutation (W217G) in the individual from an affected family. In the remaining seven sporadic patients, however, we could not demonstrate a sequence alteration. Although *NOGGIN* mutations have been demonstrated to be responsible for both multiple synostosis and proximal symphalangism syndromes, these data suggest several possibilities: 1) an alternative gene locus, 2) an alteration in the noncoding portion of *NOGGIN*, and 3) a heterozygous gene deletion.

Arthrogryposis, gastroschisis and spinal cord atresia with dysmorphic features: a variant of dyssegmental dysplasia or a new syndrome? *D.K. Manchester, E.R. Elias.* Div Genetics Services, The Children's Hosp, Denver, CO.

Case History: This 8 mo old male was the 2 kg product of a 37 wk gestation to a healthy 23 yo G3P2 mother, born by Csection. Gastroschisis was detected on prenatal ultrasound along with short limbs, club feet, and muscular hyperechogenicity. Postnatal evaluation revealed dysmorphic facies with epicanthal folds, midface hypoplasia, high arched palate, low set and dysplastic pinnae, mandibular hypoplasia, short and hyperextended neck, small thorax, gastroschisis, ileal atresia, severe shortening of all limbs with ulnar deviation of the hands, bilateral equinovarus feet deformities, and 2-4 toe syndactyly. Additional studies revealed small optic nerve heads, ventriculomegaly and increased extra axial fluid on head MRI, spinal cord thinning from T1-6, normal heart with small PFO and PDA, and normal kidneys with mild right hydronephrosis. Karyotype in amniocytes and skin fibroblasts was normal, 46, XY.

His medical course has been complicated by severe, restrictive lung disease with oxygen dependency, feeding disorder with failure to thrive necessitating Gtube feeds, and severe developmental delay. His vision is intact and hearing is normal.

Discussion: The differential diagnosis of arthrogryposis is quite broad, but few syndromes also include gastroschisis, intestinal atresia and spinal cord lesions. The association of the latter 3 problems suggests the possibility of a vascular event prenatally, compromising blood supply to the mid thorax and abdominal region. The skeletal anomalies, facial features, cardiac and renal anomalies are similar to those reported in dyssegmental dysplasia, type Silverman-Handmaker. However, the xray findings are not identical, and no abdominal wall defects or spinal cord anomalies have been reported in this skeletal dysplasia.

Genotype and phenotype analysis in patients with achondroplasia mimicking clinical features. *G-H. Kim¹, Y-L. Shin², E-J. Seo¹, H-W. Yoo^{1,2}*. 1) Medical Genetics Clinic & Lab, Asan Medical Center, Seoul, Korea; 2) Department of Pediatrics, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea.

Achondroplasia(Ach) and hypochondroplasia(Hch) are autosomal dominant skeletal dysplasia characterized by disproportionate short stature with rhizomelic shortening of the limbs. These disorders are known to be allelic and caused by specific point mutations in the fibroblast growth factor receptor 3(FGFR3) gene. Hch resembles the clinical features of Ach, but are less severe. However, the phenotype in severe cases of Hch sometimes overlaps that of Ach. In this study, we analyzed the FGFR3 gene for the previously described Ach and Hch mutations in patients with achondroplasia mimicking features and compared the clinical and radiological findings to clarify whether or not each genotype has a distinctive phenotype. Twenty six patients(14 males and 12 females) with features of Ach, such as disproportionate short stature with shortening of the limbs and large head with a prominent forehead were included in this study. Initially, Ach mutations (G380R and G375C) were screened. If both mutations were not present, subsequently Hch mutations(N540K, N540T, D540S, I538V, K650Q, K652Q) were screened by PCR-RFLP analysis or DNA sequencing. For comparison of the phenotypes, auxological parameters and radiological findings were analyzed. We detected the G380R mutation in 13 patients(50%), the N540K mutation in 5 patients(19%), and no mutations in 8 patients(31%) of 26 patients. No significant differences were found in mean age at presentation, height SDS, head circumference SDS, and sitting height to standing height ratio among three groups. Common radiological features observed in all these groups include narrowing or unchanged lumbar interpedicular distances(L1/L4 ratio), squared shortened ilia, macrocephaly, shortening of long tubular bones, long fibula and brachydactyly. Frequencies of these radiological findings were not significantly different in each group. In conclusion, it is difficult to differentiate Hch from Ach based on clinical and radiological findings, especially when presented in early childhood. Therefore, genotype-based diagnosis is needed for proper genetic counselling.

GOING BEYOND THE MARFANOID HABITUS: THE WORK-UP OF 3 UNUSUAL PATIENTS WITH SIGNIFICANT STRUCTURAL AND DEVELOPMENTAL DEFECTS IN ADDITION TO MARFANOID FEATURES. *D. Kostiner, V. Cox, A. Zanko, N. Stewart, S. Cheng, M. Golabi.* Dept. Pediatrics, Div. Medical Genetics, UC San Francisco.

Tall, thin people are often referred to genetics for evaluation of possible Marfan syndrome (MS). The DX is often ruled in/out based on H&P, so fibrillin testing is rarely needed. We present 3 patients who were referred for rule-out MS, but whose concomitant features suggested possible fibrillin defects for which FBN1 or 2 analysis might be appropriate. Pt. #1 is a 19-yo female with mental retardation (MR), seizures, deafness, marfanoid habitus, proptosis, cleft lip/palate, webbed neck, arachnodactyly and camptodactyly. Bony defects include scoliosis, vertebral defects, Sprengel deformity and craniosynostosis. Skin defects include angiofibromas, Shagreen patch and hypopigmented macules. Karyotype with subtelomeric probes reveals 46,XX. Skin findings suggest tuberous sclerosis (TS), skeletal features suggest Klippel-Feil syndrome (KFS), but overall gestalt suggests Shprintzen-Goldberg syndrome (SGS). A contiguous gene sequence is possible, but the known genes for these conditions are not adjacent (TS genes are on 9q and 16p; KFS gene might be on 8q; SGS gene might be FBN1 on 15q). Work-up could include TS mutation analysis, FBN1 mutation analysis and array-based comparative genomic hybridization (CGH). Pt. #2, born of a father-daughter mating, is a 10-yo female with dysmorphism, MR, marfanoid habitus, abnormal cranium, severe myopia, downward dislocated lenses, high palate, MVP, significant scoliosis, vertebral defects, sacral meningoceles and arachnodactyly. Karyotype reveals 46,XX. DDX includes MS, SGS and homocystinuria plus possible effects of consanguinity. Work-up could include plasma homocysteine, FBN1 mutation analysis and CGH. Pt. #3 is a 12-yo female with profound scoliosis, pectus carinatum, contractures, thin habitus, high palate and restrictive cardiomyopathy. Congenital contractural arachnodactyly is suspected. FBN2 mutation analysis is underway. We conclude that FBN1 or 2 mutation analysis might be appropriate for patients whose features are more severe than/not consistent with typical MS and in whom other diagnoses have been excluded by more accessible tests.

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Evaluating Clinical Efficacy and safety of Palmidronate in Patients with osteogenesis imperfecta. *J. Lin, S. Wang, J. Hou.* Genetic Div Pediatric Dept, Chang Gung Children's Hosp, Taoyuan, Taiwan.

Osteogenesis imperfecta (OI) is a rare genetic bone disease, which caused by the mutations within COL1A1 or COL1A2 genes. It caused osteoporosis, which would induce bone fracture and even mortality. In the past, surgical correction seemed to be the only treatment. Fifteen patients with OI had received Palmidronate 30 mg/m² every month by IV dripping for 4 hours in our hospital. They also received oral vitamin and calcium supplement once per day. Osteocalcin, alkaline phosphatase, procollagen I C-terminal peptide (PICP), collagen I telopeptide (ICTP) had been checked before treatment and every six months after treatment to evaluate the bone response and collagen fiber type I production and destruction rate. We check the bone density of every patient by DEXA in the same period as described above. It showed that every patient benefited from this treatment significantly.

Anomalies of the hand in Smith-Magenis syndrome: Evaluation by metacarpophalangeal pattern profile analysis.

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Smith-Magenis syndrome (SMS) is a multiple congenital anomalies, mental retardation syndrome associated with deletion of chromosome 17 [del(17)(p11.2p11.2)]. The clinical phenotype includes short stature, minor craniofacial anomalies, and neurobehavioral abnormalities. Brachydactyly has been described as a clinical manifestation of SMS, but measurement of the length of the bones of the hand in a large series of patients has not been performed to objectively confirm this finding. Metacarpophalangeal pattern profile analysis (MCPPPA), a method of graphic depiction of the relative size of the bones of the hand, had been used to objectively evaluate radiographs of the hand in a few patients with SMS. We measured the bones of the hand and performed MCPPPA in 29 patients with SMS. Our results demonstrated a different MCPPPA in patients with SMS than previously reported. The analysis confirmed brachydactyly and the previously described trend of more pronounced shortening of the distal bones relative to the more proximal bones, but also demonstrated a previously undescribed pattern: relative enlargement of the proximal phalanx of the thumb and middle phalanx of the fifth finger. It is interesting that our results demonstrate relative enlargement of the fifth middle phalanx, as that bone is frequently relatively shorter than the remaining bones of the hand in many syndromes. While our study objectively confirms brachydactyly in SMS and demonstrates a unique-appearing MCPPPA, statistical analysis reveals that the relative change in the size of the bones of the hand is small, implying that the MCPPPA is not highly characteristic, and correlation between the pattern for each patient and the mean pattern is not high. This suggests that MCPPPA analysis would be of limited utility in identifying patients with SMS.

Long-term survivor of malignant infantile autosomal recessive osteopetrosis with complete absence of osteoclasts and lack of response to M-CSF/RANKL. *G. Yoon*^{1,2}, *L.M. Ward*¹, *F.H. Glorieux*¹, *F. Rauch*¹. 1) Genetics Unit, Shriners Hospital for Children and McGill University Montreal, Canada; 2) Department of Medical Genetics, Alberta Children's Hospital, Calgary, Canada.

Malignant infantile osteopetrosis is an autosomal recessive disease which is usually lethal in the first decade of life. At the bone tissue level it is usually characterized by a large number of osteoclasts. We describe an unusual case of a boy with malignant infantile osteopetrosis due to lack of osteoclasts. Increased bone density, macrocephaly and hepatosplenomegaly were noted in the first year of life. A transiliac bone biopsy specimen was obtained at 10 months of age and revealed a complete absence of osteoclasts. Accordingly, serum levels of the osteoclast enzyme tartrate-resistant acid phosphatase were consistently decreased. The clinical course was further complicated by multiple fractures, blindness and compression of the left internal carotid artery. At 15 years he is severely debilitated, requires blood transfusions every 2 months and suffers from recurrent infections. Studies of the osteoclast differentiation pathways failed to reveal a treatable cause of the disease. Skin fibroblasts revealed normal production of macrophage colony-stimulating factor. The patient's peripheral blood mononuclear cells could not be stimulated to form osteoclasts in the presence of osteoprotegerin ligand and colony-stimulating factor-1. The fact that the clinical course in this patient without osteoclasts was less severe than that usually observed in children with malignant osteopetrosis suggests that the disease in these latter patients is not due solely to lack of osteoclast function.

The interparietal bone: An anatomic variation. *D.A. Staffenberg¹, J. Wu¹, A. Barkan¹, J.T. Goodrich², A. Shanske³.*
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The interparietal bone, or Os Inca, is formed by the presence of the suture mendosa. The suture is a normal variation of the human skull with no known sequelae. It was first described in 1842 in Incan skulls from Peru. The incidence is rare with estimates ranging from 0.4% to 2.5% in all races. Neither the incidence nor its pathogenesis is known in children with craniofacial anomalies. A retrospective review of 35 consecutive patients with craniofacial anomalies was done. Only 20 3-D CAT scans of the skull were available for study. Two of these demonstrated an interparietal bone. The first patient had incomplete facial duplication (diprosopus). He had a duplicated maxilla, mandible, upper frenulum and tongue. The nose was bifid and the secondary palate cleft. There were 4 infraorbital foramina and bifurcation of the upper cervical spine. There were no other sutural abnormalities. The second patient was noted to have craniosynostosis at 6 weeks of age as a result of fusion of the sagittal suture. Imaging showed a normal brain and the presence of 2 interparietal bones. The interparietal bones remain separated in most mammals and in primates fuse with the occipital bone. In man, the occipital bone is made up of a supraoccipital portion and an interparietal portion separated by the mendosal suture. Ossification and fusion of the membranous interparietal portion is complete by 20 weeks and at birth the 2 segments are fused. The mendosal suture is obliterated by 2 years. Much progress has been made in suture biology. The future study of this suture in patients with craniofacial abnormalities will result in a further understanding of sutural biology as well as the significance of the parietal bone in these patients.

Ulnar/fibular ray defect and brachydactyly type E in a family: a possible new autosomal dominant syndrome.

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The ulnar-mammary syndrome (UMS) includes postaxial ray defects, abnormalities of growth, delayed sexual development, mammillar and apocrin gland hypoplasia. The TBX3 gene for ulnar mammary syndrome has been localized to chromosome 12q23-q24.1. A gene for Holt-Oram syndrome, which presents with mostly mild symmetrical radical ray defects, associated atrial septum defect (ASD), but no lower limb malformations, has been localized to the same region of chromosome 12. Mutations in the TBX5 gene, just proximal to the TBX3 gene, have been identified in some families with Holt-Oram syndrome. Brachydactyly type E, which has not been mapped, presents with shortening of the metacarpals/phalanges in the ulnar ray in association with round face and moderately short stature. We describe a three-generation Hungarian family with variable expression of ulnar/fibular hypoplasia, brachydactyly, ulnar ray defects and short stature. The proband had ulnar hypoplasia with missing IV-Vth fingers, fibular hypoplasia on the right, bilateral club feet, growth retardation, hypoplastic midface, ASD and hemangiomas. She had normal mamillary tissue and normal sweating. Her mother had short stature, midfacial hypoplasia, hypoplastic ulna and hypoplasia of the IVth metacarpal on the right without other associated malformations. A maternal grandfather had mild bilateral fibular hypoplasia and midphalangeal brachydactyly of the IV-Vth toes. His sister had short stature and shortening of the IVth metacarpus of the left hand. We hypothesized that this family may have a mutation in the region of chromosome 12 which contains the UMS and Holt-Oram syndrome genes. However, a two-point linkage analysis of microsatellite markers spanning the locus at 12q24 did not find evidence of linkage, and linkage could be excluded for up to 10 centiMorgans for some of the markers. This family may therefore represent a previously undescribed syndrome.

An Atypical Lethal Acrofacial Dysostosis Syndrome. *B.T. Tinkle, H.M. Saal.* Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Accurate diagnosis of the acrofacial dysostoses presents with a great challenge given their rarity, phenotypic variability, and genetic heterogeneity. We report a case of a female infant born at 33 weeks gestation who died within a few hours of delivery due to respiratory failure secondary to profound retromicrognathia. The pregnancy was complicated by ultrasound diagnosis of a skeletal dysplasia at 20 weeks gestation and later by IUGR and polyhydramnios. At birth, the infant had severe respiratory distress and was very difficult to intubate. The infant died at 3.5 hours of life. She had mandibulofacial dysostosis, predominantly postaxial defects, and phocomelia of the upper extremities. The mandibulofacial dysostosis was characterized by small dysplastic ears, narrow short palpebral fissures, U-shaped cleft palate, bilateral cutis aplasia congenital lateral to the mouth, and the profound retromicrognathia. Postaxial defect was seen radiographically as missing the 5th digital ray in both hands and feet with the exception of the right foot which had a very hypoplastic 4th digital ray. The thumbs and great toes were hypoplastic as well. In addition, she had radiographic evidence of a unilateral cervical rib, 11 pairs of thoracic ribs, thin ribs, and hypoplastic scapulae. The lower limbs with the exception of the feet were relatively spared with normal appearing pelvic girdle and long bones. Chromosome study was normal. The postaxial defects may be seen in a lethal form of Miller syndrome; however, the infant did not have a lid coloboma or an extra nipple commonly seen in Miller. Further, she had synostosis of the upper extremities involving the fusion of the humerus with a single forearm bone as seen in the Rodriguez type. OMIM lists six different acrofacial dysostoses. This patient may represent a new syndrome or a further phenotypic variation of Miller or Rodriguez types of acrofacial dysostoses. The phenotypic variability and genetic heterogeneity of the acrofacial dysostoses makes diagnosis difficult especially in cases such as this one, and impacts on the genetic counseling.

New syndrome with focal dermal hypoplasia, follicular hamartomas, polysyndactyly, microphthalmia, morning glory optic disc anomaly, agenesis of the corpus callosum and polymicrogyria. *P.F. Giampietro¹, D. Babu¹, M. Koehn¹, D.M. Jacobson¹, C. Moretti¹, S.F. Patten¹, L.G. Shaffer², R.J. Gorlin³, Y. Peng⁴, W.B. Dobyns⁴.* 1) Marshfield Clinic, Marshfield, WI; 2) Baylor College of Medicine, Houston, TX; 3) Department of Oral Pathology and Genetics, University of Minnesota, Minneapolis, MN; 4) Department of Human Genetics, University of Chicago, Chicago, IL.

Multiple small areas of skin hypoplasia have been described in several genetic syndromes, including focal dermal hypoplasia (FDH), microphthalmia with linear skin defects (MLS), oculo-cerebro-cutaneous syndrome (OCCS) and terminal osseous dysplasia and pigmentary defects (TODP). All but OCCS are X-linked with increased male lethality. We describe a 13-year-old girl with an apparently new syndrome that overlaps each of these disorders. On examination, she had mild mental retardation, macrocephaly, right microphthalmos and morning glory optic disc anomaly, palmar and lip pits, polydactyly and syndactyly, swirls of skin hypopigmentation, and papular hypopigmented and herniated skin lesions reminiscent of FDH that were most prominent over her face, head, hands and feet. Brain MRI showed diffuse polymicrogyria most severe in the perisylvian and mesial frontal regions, enlarged left lateral ventricle, severe partial agenesis of the corpus callosum, and an optic nerve tumor on the right. Dermatopathologic examination of the skin lesions was consistent with basaloid follicular hamartomas. High-resolution chromosome analysis and FISH with a complete set of subtelomeric probes and several probes from the MLS region in Xp22.31 were normal. The skin and digit anomalies observed in this patient overlap with FDH, but polymicrogyria, basaloid follicular hamartomas, optic nerve tumor and morning glory optic disc anomaly have not previously been described in FDH. The skin defects in MLS are linear and the microphthalmic eyes typically have sclerocornea. Polymicrogyria has not been described. The limb anomalies in TODP are reductions rather than polysyndactyly. The skin defects are localized to the face, and digital fibromas occur rather than follicular hamartomas.

TRENDS IN THE PREVALENCE OF NEURAL TUBE DEFECTS IN NORTHEASTERN FRANCE 1979 2001.

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Objective : To examine the total prevalence of neural tube defects (NTD) and the prevalence at birth of NTD during 1979 2001 in Northeastern France. **Methods :** Using data from our registry of congenital anomalies we identified all cases of NTD (anencephaly, spina bifida and encephalocele) including live-births (LB), stillbirths and termination of pregnancies (TOP) from 1979 to 2001. Comparisons were made during 3 periods of time : 1979 1986, 1987 1994 and 1995 2001. In our country an ultrasound scan in the mid-trimester of pregnancy is routine part of antenatal care since 1979 as well as a triple test including alpha-fetoprotein since 1997, and there is no upper limit for TOP. There was no food fortification with folic acid in the area under investigation and the uptake of periconception supplementation was low, around 20% during the third period of the study, very low during the second period, and nil during the first period. **Results :** The total prevalence rates for anencephaly, spina bifida, encephalocele and all NTD in the area under investigation were not significantly different during the study periods. However there was a dramatic decrease of the prevalence rates of liveborn with NTD. No anencephalic child was born since 1988 whereas the prevalence rate per 10,000 of LB with spina bifida dropped from 4.6 during 1979-1986 to 1.5 during 1987-1994 and 1.1 during 1995-2001. The decrease of the prevalence rate of LB with encephalocele was lower. **Conclusions :** This study demonstrate that the impact of prenatal diagnosis during pregnancy on prevalence at birth of NTD was very high for anencephaly, high for spina bifida and low for encephalocele.

ARX mutation in a familial lissencephaly with agenesis of the corpus callosum syndrome. *T. Fukuda^{1,3}, J. Tohyama², N. Sugiyama³, H. Yamagata¹, K. Morohashi³, K. Kitamura⁴.* 1) Dept of Hygiene, Ehime University School of Med., Ehime, Japan; 2) Dept of Pediatrics, Okinawa Child Developmental Center, Okinawa, Japan; 3) Dept of Developmental Biol., National Institute for Basic Biol., Okazaki, Japan; 4) Mitsubishi Kagaku Institute of Life Sciences, Tokyo, Japan.

A gene for the aristaless-related homeobox (ARX) is mainly expressed in brain and considerable function of ARX protein might be the maintenance of specific neuronal subtypes in the cortex and axonal guidance in the floor plate in mouse (Miura et al., 1997). Recently, two groups showed human ARX mutations in the X-linked mental retardation families, nonspecific forms and specific forms with infantile epilepsy, myoclonic seizures and dystonia, but the brain anomalies in MRI have not been observed in these patients (Stromme et al., 2002, Bienvenu et al., 2002). We previously reported a family with X-linked lissencephaly and agenesis of the corpus callosum (the 51th ASHG, 2001). Two boys had intractable seizures and severe growth retardation, but their mother had no anomalies, suggesting that patients had an X-linked recessive condition. We have searched a region of X chromosome derived from a maternal grandfather in two affected males, and a gene for this condition was mapped to Xpter-Xp22, DXS8039- DXS710 interval, where ARX is mapped. Then, we found a missense mutation (L343Q) in exon 2 of ARX in affected siblings and their mother, but not in maternal grandparents, indicating that a fresh mutation occurred in a sperm of maternal grandfather. Our results strongly indicate that the function of ARX protein is a neural migration guidance and maintenance of specific neural subtypes in cerebral cortex. Impairment of mental development in patients with ARX mutations might be due to disturbance in neuronal cell migration.

Reciprocal fusion transcripts of two novel genes associated with absence of corpus callosum in an individual with a t(2;9)(p24;q32) translocation. *I. Grinberg, M. Ramocki, J. Dowling, D. Ledbetter, K. Millen, W. Dobyns.* Human Genetics, University of Chicago, Chicago, IL.

Agenesis of the corpus callosum (ACC) is one of the most common brain malformations in humans. ACC is a heterogeneous phenotype that may occur as an isolated malformation or as one component of a number of genetic syndromes. ACC is associated with mutations in numerous identified genes, chromosome rearrangements, and metabolic disorders. We identified a female patient with a complex phenotype that includes complete ACC, bilateral periventricular nodular heterotopia (PNH), and bilateral chorioretinal and iris colobomas. Examination of the patient also showed frontal bossing, left ptosis, dysplastic left ear, right frontal hemangioma, capillary hemangioma on the right side of the vulva, strawberry nevi on the left knee and shoulder, bilateral leukocoria, hearing loss, ventricular septal defects, vesicoureteral reflux, and hyperreflexia of all four extremities. She was born to a diabetic mother and the identification of mild concentric left ventricular hypertrophy was attributed to this fact. Karyotype analysis on peripheral blood lymphocytes of the patient identified a balanced, reciprocal, de novo chromosome translocation t(2;9)(p24;q32) in the proband but not in her biological parents. Physical mapping by FISH analysis of the translocation breakpoint lead to the identification of two novel zinc-finger-encoding transcripts that are disrupted in this patient. One of these genes encodes an ASX-related transcription factor, the homologue of which has a role in Polycomb gene regulation in *Drosophila*. The other gene encodes GT4-2, a mouse homologue of which was initially isolated in one of the first gene trap screens in mouse ES cells. In mouse, both genes are ubiquitously expressed in the developing CNS. Interestingly, reciprocal fusion in-frame transcripts are generated from each gene in patient lymphoblasts. Thus, the patient phenotype is caused by either loss of one or both gene(s) and/or gain of function of one or both fusion transcript(s).

Hypogonadism in familial cerebellar ataxia with muscle coenzyme Q₁₀ deficiency. *R. Freeman¹, A. Naini², S.*

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Coenzyme Q₁₀ is a component of the electron-transport chain that transfers electrons from complex I and II to complex III. A recently described disorder, familial cerebellar ataxia with coenzyme Q₁₀ deficiency is characterized by familial ataxia associated with pyramidal signs, seizures, cerebellar atrophy and dementia. We recently identified a 41 year old woman with this disorder and hypogonadotropic hypogonadism. She developed normally until 2 years when she developed ataxia and nystagmus and began losing milestones. A seizure disorder began at 2 years. An MRI showed marked cerebellar and pontine atrophy and a diffuse disorder of myelination. A muscle biopsy showed normal mitochondrial enzyme activities and normal muscle morphology. CoQ₁₀ levels in muscle and serum were very depressed. An endocrine evaluation done at age 19 revealed hypogonadotropic hypogonadism. Pituitary function was normal including LH, TRH and ACTH stimulation testing. Her three sibs, 2 sisters and a brother, have had a similar clinical course. The parents are unrelated and the 3 of the 4 sibs who have been tested all have normal pituitary function. At the present time, she is non-verbal and non-ambulatory. Her 38 year old sister was noted to have a questionable response to therapeutic doses of CoQ₁₀. Idiopathic hypogonadotropic hypogonadism (IHH) is a genetically and clinically heterogeneous group of disorders. When associated with anosmia, it is known as Kallmann syndrome (KS). In its X-linked form, it is caused by mutations in the KAL gene. Mutations in the KAL gene occur in only 14% of familial cases of KS. The majority of familial cases are normosmic and are caused by at least 2 presently unidentified autosomal genes. We suggest that an error in metabolism of CoQ₁₀ might also be responsible for familial IHH. The complexity of CoQ₁₀ biosynthesis suggests that different enzyme defects may cause different clinical syndromes.

Further delineation of Stuve-Wiedeman Syndrome in survivors . *LI. Al-Gazali¹, A. Ravenscroft², A. Feng³, A. Shubbar⁴, A. Al-Saggaf⁵, D. Haas⁶*. 1) Paediatrics, FMHS, UAE University, Al Ain, Abu Dhabi, UAE; 2) Tygerberg Hospital and University of Stellenbosch, Department of Paediatrics and Child Health, Republic of South Africa; 3) Sheikh Khalifa Medical Center, Department of Paediatrics, Abu Dhabi, UAE; 4) Corniche Hospital, Department of Paediatrics, Abu Dhabi, UAE; 5) Al Jazeirah Hospital, Department of Paediatrics, Abu Dhabi, UAE; 6) Tawam Hospital, Department of Radiology, Al Ain, UAE.

Stuve-Wiedeman syndrome (SWS) is an autosomal recessive bone dysplasia characterized by camptomelia, contractures of the big joints and camptodactyly. The clinical course is complicated by hyperthermic episodes, respiratory insufficiency, feeding & swallowing difficulties and distinct radiological changes. It is usually lethal as most of the reported cases died in the first year of life. We report 3 children from 2 inbred Arab families with Stuve-Wiedeman syndrome who have survived the first 2 years of life (ages are 6 years, 2.8 years and 20 months). All exhibited a specific phenotype very similar to that described by Chen et al 2001. In all 3 children the skeletal abnormalities progressed to severe bowing of the long bones with prominent joints and severe spinal deformity. Neurological symptoms were present in all of them. These included temperature instability with excessive sweating, reduced pain sensation with repeated injury to the tongue and limbs absent corneal reflexes and smooth tongue. Mentality was normal in all of them. There were specific radiological changes which included undertubulation of the diaphyses, rarefaction and striation of metaphyses, destruction of the femoral heads and spinal deformity. We suggest that survival in this syndrome is possible and that prognosis improve immensely after the first year of life. This should be taken into consideration when counselling parents of affected children. This report further support the existence of a specific phenotype in SWS survivors which include, in addition to the skeletal abnormalities and distinctive radiological features, unique neurological symptoms reminiscent of dysautonomia.

Unilateral Epidermal Nevus, Epidermolytic Hyperkeratotic Type, Following Blascko Lines Associated with Contralateral Hemihypertrophy, Hemimegalocephaly, Pachygyria and Developmental Delay. *C. Gutierrez¹, F. Suarez¹, J.C. Prieto^{1,2}*. 1) Instituto de Genetica Humana, Universidad Javeriana, Bogota, Colombia; 2) Hospital la Victoria, Departamento de Genetica, Bogota, Colombia.

Epidermal nevus is a benign and congenital hyperplasia of epidermis that follows the Blaschko lines, characterized by located or systematized verrucous plaques that display histopathologic features typical of epidermolytic hyperkeratosis. Hemihypertrophy, hemimegalocephaly and ipsilateral gyral malformations are related with the nevus sebaceous of Jadassohn, not with the epidermal nevus alone. The central nervous system malformations are also related, if both kinds of skin lesion are found in the same patient (Solomon Syndrome). We report a 1 year old masculine patient, first child of a healthy no consanguineous parents, with a epidermal nevus, following the blaschko lines, confirmed with histopatological analysis that showed features of epidermolytic hyperkeratosis and discarding a nevus of Jadassohn, associated with other systemic malformations. The skin lesion consist of verrucous plaques in the left side of the lower face, neck, and thorax, following blascko lines, not crossing the middle line and a complete right body hemihypertrophy including face and head and a severe development delay. Cerebral magnetic resonance showed a completed right hemimegalocephaly, associated with a severe neuronal migration disorder presented as a pachygyria. To the best of our knowledge, no similar case observations have been recorded with the presented neurocutaneous disorder and the presence of epidermal nevus alone not associated with nevus sebaceous. This new case contributes with new clinic and radiological findings that have not been reported on previous cases.

Identification of the gelsolin Asp187Tyr mutation in a French family with familial amyloidosis of the Finnish type (FAF). *N. Chastan*¹, *S. Baert*², *P. Saugier-veber*¹, *D. Hannequin*^{1, 2}, *T. Frebourg*^{1, 2}. 1) Department of Neurology, CHU de Rouen, 76031 Rouen, France; 2) Department of Genetics, CHU de Rouen, 76031 Rouen, France.

Familial amyloidosis of the Finnish type (FAF) is an autosomal dominant disease resulting from the accumulation of a 71-amino acid amyloidogenic fragment derived from mutant gelsolin. FAF is characterized by corneal lattice dystrophy, progressive cranial and peripheral neuropathy, and skin changes. Two gelsolin mutations have been described so far : the most common, identified in all Finnish families, in two American and three Japanese families is a G654A transition leading to an Asp to Asn substitution at residue 187 of the protein. The second mutation is a G654T transition resulting in a Asp187Tyr substitution and was found in a Danish and a Czech family. Here we report the first case of a French FAF family which originated from Normandy. The index case developed at 55 years distal arm numbness, weakness of the facial muscle and tongue responsible for speech difficulties. Clinical investigation showed diminished touch and pain senses with reduced vibratory perception, atrophic tongue with fasciculations, right facial weakness, right impaired hearing, paresthesia of the fifth cranial nerve, dysphagia and dysarthry. The slit lamp examination showed bilateral corneal dystrophy. His mother and grand-mother were both affected, with bilateral blepharochalasis, abnormally slack and hanging skin over the face, pronounced bilateral facial weakness, atrophic tongue with fasciculations. Sequencing analysis of the gelsolin gene exon 4 identified the Asp187Tyr mutation in the proband and his mother. Investigations are under process to determine if this family shares a common haplotype with the Danish family, which would confirm the existence of a founder effect.

Evidence for Genetic Heterogeneity of the Walker Warburg Syndrome, a Disorder of Neuronal Migration. *S.*

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Human genetics has proven to be a powerful method for identifying genes involved in cortical neuronal migration. Walker Warburg Syndrome (WWS), characterized by hydrocephalus, agyria, retinal dysplasia, encephalocele and congenital muscular dystrophy demonstrates over migration of neurons through the pial surface. Cloning of the WWS gene or gene(s) should reveal mechanisms that arrest migrating neurons. With this aim we performed a genome-wide linkage analysis screen of ten consanguineous pedigrees with WWS. The screen revealed several promising loci including 16q12, 17q11, 9q34 and 14q24. Amplification of additional microsatellite markers at these loci confirmed identity by descent in a subset of patients. However none of these loci achieved a LOD score of greater than 3.0 and no single locus was identified as linked in all pedigrees. Our results strongly suggest that WWS is genetically heterogeneous. In collaboration with the lab of Dr. Han Brunner, we have been sequencing candidate genes for mutations and have preliminary evidence that, as with muscle eye brain disease, o-mannosyl transferase genes are mutated in WWS.

Action Myoclonus-Renal Failure Syndrome: Characterization of a novel autosomal recessive disease. A.

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Action myoclonus-renal failure syndrome (AMRF) is a distinctive form of progressive myoclonus epilepsy associated with severe renal dysfunction. The first and only description of AMRF was in 4 French-Canadian patients (*Adv Neurol*,1986). We describe 15 patients (11 new) in 9 families from 5 countries. Autosomal recessive inheritance was determined based on absence of affected cases in previous generation; multiple affected siblings (4 families); parental consanguinity (3 families); and parental origins from the same rural area (6 families). Initial presentation can be renal or neurological or both. Tremor (onset:17-26 years, mean:19.8), progressive action-myoclonus (onset:19-29 years, mean:21.7), infrequent generalized seizures (onset:20-28 years, mean:22.7) and cerebellar features are characteristic. Proteinuria was detected in all cases and was detected between 10-30 years, and progressed within 8 years to renal failure in 12 patients. Brain autopsy (2 patients) revealed extra-neuronal pigment accumulation. Renal biopsy showed collapsing glomerulopathy. Our study extends the original AMRF phenotype and demonstrates that it is not due to a private French Canadian gene. Independent progression of neurological and renal symptoms suggests pleiotropic effects of a unitary molecular lesion, differentially affecting the two organ systems. Treatment with anti-myoclonic drugs and effective dialysis and/or transplantation can improve quality of life. The renal lesion in AMRF is a recessive form of collapsing glomerulopathy. Genes identified for focal segmental glomerulosclerosis to date are thus good candidates.

Obsessive compulsive symptoms in parents of individuals with Autistic Disorder. *R.K. Abramson¹, K.M. Wieduwilt¹, S.A. Ravan¹, K.A. Decena¹, H.H. Wright¹, M.L. Cuccaro².* 1) Dept Neuropsych & Behav Sci, W.S. Hall Psychiatric Inst, Columbia, SC; 2) Center for Human Genetics, Duke University, Durham, N.C.

One area of impairment in Autistic Disorder (AD) is repetitive behaviors and stereotyped patterns. Gordon(1992), Bolton(1998) and Piven(1999) asked if restrictive and repetitive behaviors seen in persons with AD are related to symptoms of Obsessive Compulsive Disorder(OCD). Cook(1994)and Bolton(1998)found an increase in OC symptoms/OCD in family members.This study examines OC symptoms in parents of AD individuals as a first step in relating OC symptoms in parents and restrictive/repetitive behaviors in AD individuals. The Yale-Brown Obsessive Compulsive Behavior Scale(Y-BOCS;Goodman,1986) a self-report scale that identifies the nature and extent of OC symptoms was sent to parents in the Duke/USC AD research project. Males(n=28) were 45.2% of the sample and females(n=34)54.8%. Clinically significant Y-BOCS scores were present in 34% of parents, with moderate to severe scores in 13.2%. Family history data for 131 families at USC was similar: 34.4% of families had first/second degree relatives with probable/ confirmed OCD and 11.5% with confirmed OCD. Parents with clinical Y-BOCS scores differed significantly from those with subclinical Y-BOCS scores, endorsing certain obsessions and compulsions most or all of the time. Significant obsessions were fear of doing something embarrassing(p=0.014);fear of being responsible for something terrible happening(p=0.010); bothered by certain sounds/noises (p=0.005); and overly concerned with body parts and/or appearance(p=0.035). Significant compulsions were check locks, stove, appliances(p=0.001);check you did not make a mistake(p=0.001); and reread/rewrite things (p=0.035). Significant compulsions and rituals in this study compare to Cuccaro's (2000) Factor 2 derived from factor analysis of restrictive/repetitive behaviors from the Autism Diagnostic Interview-Revised. Factor 2 reflects problems with minor changes, compulsions and rituals in AD individuals. Future correlation of AD Factor 2 scores with clinical parent OC symptoms could clarify commonality of OC symptoms between parents and AD children and identify a subset of AD families.

Classic Rett syndrome in a boy with MECP2 mutation. *I. Kondo¹, T. Masuyama², S. Matsuo³, K. Kitsuki², Y. Kan², K. Ishii³, H. Yamagata¹.* 1) Dept. of Hygiene, Ehime Univ. School of Medicine, Ehime, Japan; 2) Saga Seishi Child Developmental Medical Center, Saga, Japan; 3) Dept. of Pediatr., Saga Medical School, Saga, Japan.

Rett syndrome (RTT) is a neurodevelopmental disorder affecting females in a sporadic manner and DNA mutations in methyl-CpG binding protein 2 gene (MECP2) have been detected in about 80% of the female patients with classical RTT. The existence of RTT males has been discussed extensively, because males with MECP2 mutations have severe encephalopathy in families with female RTT patients. In addition, MECP2 mutations have been detected in males with an X-linked recessive form of nonspecific mental retardation syndromes. Classic RTT was diagnosed in two boys with a somatic mosaicism of MECP2 mutation, 2 bp deletion within exon 3 and R133H. We report here a family with a missense mutation in MECP2, R133C. A female patient was diagnosed with RTT at age 3 and MECP2 mutation, R133C was confirmed at age 12. Developmental delay was noted in her young brother at age 12 months, and classic RTT symptoms were developed at age 3. MECP2 analysis was performed at the age 10. He had only a C133 allele indicating without somatic mosaicism. Their mother was a carrier for the R133C and she was slightly mentally retarded. The R133C mutation has been detected in female patients with classic RTT and preserved speech variant form of RTT, but not in male patients with nonspecific X-linked mental retardation syndromes. These results strongly suggest that a phenotype caused by DNA mutations in MECP2 is determined by position of mutation site and the R133 is a critical amino acid residue to introduce RTT symptoms in human.

A sibling of a patient with Smith-Lemli-Opitz syndrome was a heterozygote for DHCR7 mutation. *M. Mori, M. Komiyama, S. Kato, L. Hong, T. Yamagata, M. Mizuguchi, M.Y. Momoi.* Dept Pediatrics, Jichi Medical Sch, Tochigi, Japan.

SLOS is an autosomal recessive disorder that is caused by the reduced activity of 7-dehydrocholesterol B7-reductase(DHCR7). Low plasma and tissue cholesterol levels and increased plasma and tissue 7-dehydrocholesterol (7-DHC) are characteristic biochemical findings. Because of the importance of cholesterol in morphogenesis, the derangement of cholesterol metabolism causes varieties of congenital anomalies as observed in SLOS. An infant presenting with failure to thrive, generalized hypotonia, and developmental delay was diagnosed as having SLOS with the serum cholesterol level of 30mg/dl. Her anomalies included mild facial dysmorphism, prominent foreheads, syndactyly in her 2, 3 toes, ambiguous genitalia and pyloric stenosis. Sequence analyses of DHCR7 revealed that she was a compound heterozygote with G303R and R352Q. The former was a novel missense mutation found in SLOS. The patient's sister who had holoprosencephaly without associating other anomalies and with the normal level of serum cholesterol was a heterozygote with G303R. The SLOS is rare in Asian population and this is the first report that confirmed DHCR7 gene mutations in Japanese patient. The patient's clinical symptoms and biochemical characters were typical in SLOS. R352Q mutation that is located in the transmembrane domain (TM mutations) was related with the mildest clinical phenotype. A novel missense mutation, G303R, substitutes a highly conserved amino acid at 303 and is also located in TM domain. Considering the severe clinical and biochemical phenotype of our patient, this novel mutation can cause profound defect in the enzyme. Whether G303R detected in her sibling can cause holoprosencephaly or requires other gene mutation, the further studies is required.

Spinocerebellar ataxia type 2 (SCA2) presenting with ophthalmoparesis and developmental delay in infancy. *P. Moretti¹, M. Blazo¹, L. Garcia², F. Scaglia¹*. 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030; 2) Pediatrics and Neurology, UT San Antonio, Mc Allen, TX 78503.

Autosomal dominant cerebellar ataxias are a heterogeneous group of neurodegenerative disorders that generally present in adulthood. SCA2 typically presents with progressive cerebellar symptoms and slow ocular saccades. An 11-year-old boy was evaluated for progressive ataxia, ophthalmoparesis, and developmental delay. Abnormal eye movements were noted at age two months. Developmental delay was noted at age six months. At seven years he was found to have poor balance and coordination and progressive cognitive deterioration. He has developed difficulty swallowing and loss of bladder function. The mother was unaffected and the father was not known. General physical examination was normal. On neurological examination he had bilateral external ophthalmoplegia, ataxic dysarthria, dysmetria and tremor in the upper extremities, and marked gait ataxia. There was no evidence of pigmentary retinopathy. Brain MRI demonstrated cerebellar, brainstem, and cerebral atrophy. Brain MR spectroscopy, plasma amino acids, and urine organic acids were normal. Alpha-fetoprotein and immunoglobulin levels were normal. An ataxia panel showed 62 repeats in one of the alleles of the SCA2 gene. The mother had normal SCA2 alleles with 22 repeats (normal size between 14 and 31 CAG repeats). The patient was diagnosed with SCA2 based on history, examination findings, and expansion of CAG repeats in one allele of the SCA2 gene. Most cases of SCA2 present between 20 and 40 years and affected individuals have between 34 and 57 CAG repeats. Ocular motor apraxia or ophthalmoparesis may be present in early stages of the disorder. Infantile and childhood cases of SCA2 have very rarely been described in the literature. The most severe case known was described in a neonate with over 200 CAG repeats. This case contributes to the characterization of the phenotypic spectrum of SCA2 and highlights the importance of considering this diagnosis in young children. Developmental delay in addition to abnormal eye movements appears to be an early clinical feature in this age group.

Outcomes of a genetic and neurological evaluation in 84 individuals with pervasive developmental disorders. *A.P. Marques-de-Faria¹, C.E. Steiner¹, M.M. Guerreiro²*. 1) Departamento de Genética Médica; 2) Departamento de Neurologia, FCM-UNICAMP, Campinas,SP, Brazil.

Pervasive developmental disorders (PDD) constitute a heterogeneous group of conditions comprising autism, atypical autism, Asperger syndrome, Rett syndrome, and PDD not otherwise specified. There is a wide range of phenotypic expression and its management presents particular challenges for clinicians. We studied 103 individuals with a preliminary diagnosis of autism using a protocol that included an initial assessment based on neuropsychological criteria, followed by a careful clinical evaluation with special attention to dysmorphic and neurological findings, screening for inborn errors of metabolism, chromosomal analysis, molecular study of FRAXA, FRAXE and FRAXF mutations, EEG, magnetic resonance and SPECT imaging. As 2 patients had the diagnosis of Rett syndrome, 6 had other functional neuropsychiatric diagnosis and 11 were excluded due to incomplete complementary investigation, the final sample comprised 84 individuals (71 males and 13 females) who met the DSM-IV criteria for PDD and were classified in 3 main clinical groups: autism (71.4%), atypical autism (15.5%) and Asperger syndrome (13.1%). A recognizable syndrome or genetic disorder was identified in 17 individuals (20.2%), of which 3 with Down syndrome (3.6%), 2 with untreated phenylketonuria (2.4%), 1 with fragile X syndrome (1.2%), 1 with tuberous sclerosis (1.2%), 5 with other monogenic conditions (6%) and 2 with balanced chromosomal rearrangements (2.4%), one maternal chromosome 9 pericentric inversion and one Robertsonian translocation (15q21q), besides 3 individuals with non-genetic etiologies. The relatively high prevalence of genetic causes corroborates previous data that pointed individuals presenting PDD should have a detailed clinical genetic evaluation, as well as chromosome analysis and tests for the fragile X mutations. Neuroimaging findings revealed low frequency and specificity among the groups and the value of these exams as routine in all patients with PDD seems secondary. Other aspects as sex ratio, familial recurrence and some findings as increased head circumferences are also discussed.

Neuronal intranuclear inclusions in a tremor/ataxia syndrome among fragile X premutation carriers. *P.J.*

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Fragile X syndrome is generally regarded as a non-progressive neurodevelopmental disorder in which premutation carriers (~55 to 200 CGG repeats; FMR1 gene) are largely unaffected. However, neurological findings of progressive action tremor, ataxia, cognitive decline, and generalized brain atrophy have recently been described in some adult males with premutation alleles. Neurohistological studies on the brains of five adult (>50y) male carriers (range: 70-135 CGG repeats) who had the neurological findings reveal ubiquitin-positive intranuclear inclusions in both neuronal and astrocytic cells of all five males, with highest frequencies (~40% of neuronal nuclei) in the hippocampus. These observations strongly support the association of inclusions with the premutation alleles. Additional studies were also conducted on a 75y female with the premutation but without any neurological findings, a cognitively impaired adolescent female with the premutation, and a typically affected young adult male with the full mutation. None of these patients displayed inclusions or other neuropathologic findings. The basis for inclusion formation among premutation carriers is not known. The FMR1 protein (FMRP) is structurally normal and is present at near-normal levels in the low-mid premutation range. Moreover, the inclusions do not react to antibodies to tau protein, alpha-synuclein, or polyglutamine repeats, suggesting a novel pathogenic mechanism. FMR1 mRNA levels are elevated by 2 to 5-fold in this range, raising the possibility that altered message levels may participate in inclusion formation and neuropathology.

Monosomy 1pter Syndrome: A Case Report. *C. Li*^{1,2}, *C. Prasad*^{1,2}. 1) Section Genetics and Metabolism, Department of Biochemistry and Medical Genetics, University of Manitoba; 2) Pediatrics and Child Health, Health Sciences Center, Winnipeg, R3A 1R9, Canada.

We report a 20 year old male patient with inherited unbalanced karyotype 45,XY, der(1)t(1;15)(p36.3;q11),-15mat. The mother carries the balanced reciprocal t(1;15) and is phenotypically normal. The proband has lost a tiny segment of chromosome 15pter~q11. As loss of this segment of chromosome 15 is clinically inconsequential, our patient is primarily monosomic for 1p36.3 only. Hemizyosity for 1pter has the patient exhibiting profound mental retardation, no speech and severe physical handicap (wheel-chair bound, ambulatory only by means of crawling). He also has craniofacial dysmorphism with flat occiput, sloping forehead, prominent supra-orbital ridges, deep-set eyes, malar hypoplasia and prognathia. In addition, he has patches of discoloration of the scalp hair, although examination of the eyes and skin did not reveal heterochromia and hyper- or hypo-pigmented patches or streaks. Delineation of chromosomal disorders in patients with unbalanced karyotypes is often complicated by the fact that the patients are often monosomic for one chromosome segment and trisomic for another chromosome segment that are involved in an originally balanced rearrangement. Therefore, in those situations, it is very hard, if possible at all, to attribute observed phenotypes to a particular imbalance, be it segmental monosomy or segmental trisomy. Our case, therefore, may be valuable in correlating cytogenetic abnormality and clinical phenotypes. For example, certain features of del(1pter) described in the literature were observed in our patient. Unique features, however, were also appreciated. Furthermore, the nature of the chromosomal anomaly in our patient may be taken advantage of to elucidate the molecular basis of these patients' deviation from normal development: known cell cycle and neuro-transmitter receptor genes, e.g. CDC2L1(mim#176837), CDC2L2(mim#116951), and GABRD(mim#137163) are located at 1p36.3. Haplo-insufficiency and/or dominant negative effects of these genes may each, and in combination, have their influence on phenotypic expressions.

Identifying genetically informative phenotypes in autism spectrum disorders (ASD). *P. Szatmari¹, C. Merrette², M. Jones³, R. Palmour⁴, L. Zwaigenbaum⁵, M. Roy², M. Maziade².* 1) Dept Psychiatry, McMaster Univ, Hamilton, ON, Canada; 2) Dept of Psychiatry, Laval Univ, Quebec City, Quebec, Canada; 3) Dept of Behavioral Sciences, Penn State Univ College of Medicine, Hershey PA, USA; 4) Dept of Psychiatry, McGill Univ, Montreal Quebec, Canada; 5) Dept of Pediatrics, McMaster Univ, Hamilton ON Canada.

Even though autism is a highly heritable disorder, it has proven difficult to identify susceptibility genes. One possible reason for this is that genetically informative phenotypes have not been used in current studies. Autism is a clinically heterogeneous disorder but most studies have not taken a systematic approach to narrowing the phenotype. We followed a logical series of steps to identify more informative phenotypes using a sample of 57 affected sib pairs with autism or another ASD and 45 singletons. Data included measures of autistic symptoms using the ADI-R, measures of level of functioning, IQ on unaffected first degree relatives, and a measure of PDD like traits (the extended autism phenotype) in other family members. In the first step, a factor analysis revealed that the ASD phenotype is composed of two separate dimensions; autistic symptoms and level of functioning (LOF). In the second step, a cluster analysis showed that two more homogeneous subgroups can be identified that essentially differ on LOF; a higher functioning group with autism, Asperger syndrome and PDDNOS and a lower functioning group with just autism. This classification was familial in that proband and affected sibling tended to be in the same cluster (high or low functioning). In the third step, we found that the high functioning cluster appeared to be more familial than the low functioning one since it was associated with a higher risk of the extended autism phenotype. These data suggest that the phenotype to be used in genetic studies should include higher functioning probands and affected siblings. The low functioning cluster may represent an admixture of non-specific mental retardation syndromes. Including a large number of these lower functioning sib pairs in genome scans may make it difficult to then identify susceptibility genes.

Tumor burden and neurological symptoms in neurofibromatosis 2. A. Shenton¹, D.G.R. Evans¹, J.M. Friedman², M.E. Baser³. 1) Department of Medical Genetics, St. Mary's Hospital, Manchester, U.K; 2) Department of Medical Genetics, University of British Columbia, Vancouver, B.C., Canada; 3) Los Angeles, CA, U.S.A.

Neurofibromatosis 2 (NF2) is an autosomal dominant disease that is characterized by nervous system tumors. Vestibular schwannomas (VSS) are the most common type of tumor, but intracranial meningiomas, spinal tumors, non-VS cranial nerve tumors, and peripheral nerve tumors are also very common. We used data from the United Kingdom NF2 registry to examine the association between non-VS nervous system tumors and non-vestibular neurological symptoms in 227 people from 151 families (120 new mutations and 107 inherited cases) who were personally examined by D.G.R.E.; known somatic mosaics were excluded. The prevalence of VSS was 95%. The prevalence of non-vestibular symptoms was mononeuropathy, 11%; headache, 29%; seizures, 20%, paraesthesias, 27%; wasting or weakness, 33%; peripheral neuropathy, 15%; and facial palsy, 47%. Multiple logistic regression was used calculate relative risks (RR). The RR of mononeuropathy decreased 0.86-fold per year increase in age at onset of symptoms (95% confidence interval (CI), 0.80-0.91). The RR of headache was 1.98-fold greater in people with non-VS cranial nerve tumors than in those without, and 1.99-fold greater in people with intracranial meningiomas than in those without (95% CI, 1.06-3.72). The RR of seizures increased 1.26-fold per intracranial meningioma (95% CI, 1.08-1.47). The RR of paraesthesias increased 1.15-fold per peripheral nerve tumor (95% CI, 1.07-1.24) and was 3.22-fold greater in people with intracranial meningiomas than in those without (95% CI, 1.72-5.41). The RR of wasting or weakness increased 1.22-fold per intracranial meningioma (95% CI, 1.05-1.41). The RR of peripheral neuropathy increased 1.09-fold per peripheral nerve tumor (95% CI, 1.02-1.16). The RR of facial palsy was 2.31-fold greater in people with non-VS cranial nerve tumors than in those without (95% CI, 1.29-4.14). In future work, we will examine the relationship between age at onset of symptoms and age at diagnosis of tumors.

EEG abnormalities and epilepsy in Smith-Magenis syndrome [del(17)(p11.2p11.2)]. *L. Potocki*^{1,5,6}, *J.K. Lynch*^{2,3,5}, *D.G. Glaze*^{2,3,4,5,6}, *K. Walz*^{1,5}, *J.L. Noebels*^{2,5}, *J.R. Lupski*^{1,4,5,6}. 1) Department of Molecular and Human Genetics; 2) Peter Kellaway Section of Neurophysiology; 3) Department of Neurology; 4) Department of Pediatrics; 5) Baylor College of Medicine, Houston, TX; 6) Texas Children's Hospital, Houston.

Smith-Magenis syndrome (SMS) is a syndrome of multiple congenital anomalies and mental retardation associated with a deletion of chromosome 17p11.2. The neurobehavioral phenotype in SMS includes sleep disturbance, aggressive and self-injurious behaviors, EEG abnormalities, and seizures. Sixty patients with SMS were evaluated as part of a multidisciplinary clinical protocol at the Texas Childrens Hospital. Prolonged EEGs were obtained during an overnight sleep study. Of the 60 patients, 29 (48.3%) had abnormal EEGs, 27 of these were epileptiform in nature. Focal abnormalities were found in 8/27 (29.6%), and 21/27 (77.8%) showed generalized epileptiform features, including various 2-4 cps spike and slow wave patterns in single waveforms or bursts up to several seconds in duration. Seizures were reported in only 11 (18.3%), and included absence (7/11), generalized tonic-clonic (4/11), and drop attacks (2/11). Half of this group (54.5%) showed abnormal EEGs. Human chromosome 17p11.2 is syntenic to the 32-34 cM region of murine chromosome 11. Interestingly, we found that EEG abnormalities and/or seizures are present in 14-29% of mice (strain-dependent) deleted for the syntenic region of SMS. Epilepsy is a frequent component of SMS. In our study, nearly one-half of patients with SMS have abnormal EEGs, the majority of which are generalized spike and slow wave variants, however, the epileptiform EEG abnormalities do not correlate well with seizure history, which was positive in 11/60. The common SMS interval spans ~3-4Mb. The deletion size does not correlate with the presence of EEG abnormalities or seizures. None of the genes identified within the commonly deleted region have a currently known role in epilepsy. The mouse model will be useful in characterizing the contribution of specific genes to the epilepsy of SMS.

A clinical study of the Troyer syndrome, a recessively inherited form of complicated hereditary spastic paraplegia. *C. Proukakis*^{1,2}, *H. Cross*³, *H. Patel*¹, *M.A. Patton*¹, *A. Valentine*⁴, *A.H. Crosby*¹. 1) Department of Medical Genetics, St George's Medical School, London, England; 2) Department of Clinical Neurosciences, Royal Free and University College Medical School, London, England; 3) Department of Ophthalmology, University of Arizona, Tucson, Arizona; 4) Department of Neuroradiology, Royal Free Hospital, London, England.

The Troyer syndrome is a recessively inherited form of complicated hereditary spastic paraplegia with distal amyotrophy. It was originally described in 1967 in an Old Order Amish population practicing strict endogamy over a number of generations. A few similar cases have been described since around the world. We have performed a detailed clinical study of Troyer syndrome in the Amish population. A total of 21 patients were examined, including 3 from the original study. The cardinal features were spastic paraparesis with upper limb hyperreflexia, dysarthria and distal amyotrophy. Early milestones were delayed, and difficulties at school were reported. Gait and speech were abnormal from the onset. Emotional lability was usually present and the disease was slowly progressive. Mild cerebellar signs were often found, and the most severe cases had choreoathetoid movements. Skeletal abnormalities were also present including pes cavus, short stature and joint hyperextensibility. Blood testing for metabolic disorders was unremarkable. MRI scans in 3 patients revealed white matter hyperintensity in various parts of the brain including periventricular areas (especially temporoparietal), the internal capsules and the cerebellar peduncles. Nerve conduction studies and electromyograms in 2 patients were unremarkable. Parallel gene identification studies of these patients have led to the discovery of a novel gene designated spartin (also presented at this meeting).

Sensory neuropathy with decreased NCV in Fabry patients with preserved renal function. *P. Ribai¹, M. Henri², F. Dehout², D. Roland¹, L. Van Maldergem¹.* 1) Ctr Human Genetics, Inst Pathology and Genetics, Lovreval, Belgium; 2) Dpt of Neurology and Nephrology, ISPPC, Charleroi, Belgium.

The involvement of large myelinated fibers has remained controversial in the pathophysiology of peripheral neuropathy associated with alpha-galactosidase deficiency. If involvement of c-fibers has been clearly demonstrated, alterations in nerve conduction velocities (NCV) and/or amplitude of action potentials of large fibers have been usually attributed to renal insufficiency which is a classical complication in Fabry disease. We have undertaken a systematic motor and sensitive evaluation of a group of eleven Fabry disease's patients, in which three were untreated and eight were under enzyme replacement therapy. Upper and lower limbs NCV were measured by standard methods, at the same skin temperature. Sural, external and internal peroneal nerves were tested in the lower limbs. One untreated patient (a symptomatic female carrier) had a decrease in sural nerve conduction velocity and amplitude of sensory action potential. Her kidney function was normal. Six patients under enzyme replacement therapy had abnormal amplitudes and/or NCV in lower limbs. Interestingly, only three of these six patients had renal insufficiency. These results suggest that a large fibers sensory neuropathy independent from renal insufficiency is present in Fabry patients.

Diprosopus: A unique case and review of the literature. *J. Wu*¹, *A. Shanske*², *D. Staffenberg*¹, *J.B. Mulliken*³. 1) Plastic Surgery, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY; 2) Center for Craniofacial Disorders, Children's Hospital at Montefiore, Albert Einstein College of Medicine, Bronx, NY; 3) Craniofacial Centre, The Children's Hospital, Harvard Medical School, Boston, MA.

Craniofacial duplication, or diprosopus, is considered an unusual variant of conjoined twins. The phenotype comprises a wide spectrum and ranges from partial duplication of a few facial structures to complete dicephalus. These cases are rare and differ sufficiently from one another to make each case unique and worthy of study for their contribution to our understanding of pathogenesis and management. We recently evaluated a male infant with partial duplication. BS was the 2845gm product of a term uneventful pregnancy. The newborn examination revealed low-set pinna, orbital hypertelorism, a widow's peak, and downward obliquity of the palpebral fissures. The forehead had prominent transverse wrinkling. Cupid's bow was wide and the nasal tip was bifid. The maxilla and mandible were duplicated and there were 2 tongues with a common base. The upper labial frenulum was duplicated and the secondary palate was cleft. Imaging revealed bifurcation of the upper cervical spine, absence of the corpus callosum, and abnormal olfactory gyri. The midbrain was dysmorphic, the medulla and clivus were partially split and the pituitary gland and stalk were duplicated. A separate interparietal bone was noted. His postnatal course was complicated by chronic respiratory distress secondary to secretions resulting in a gastrostomy at 3 months. Experimental and embryologic evidence suggests the importance of notochord formation in the development of facial duplication. The notochord process grows between ectoderm and endoderm until it reaches the prochordal plate. It elongates and eventually extends to the oropharyngeal membrane by about 20 days. The temporal-spatial relationships of its development predicts the common involvement of the forebrain, mouth, mandible, maxillae, tongue and thyroid. It is hoped that this case and future cases as well as discoveries in notochordal signalling pathways will result in a clearer understanding of the pathogenesis of this defect.

Autism: Characterization of Sleep Disorders. *P.T. Yu, T.N. Takahashi, J.H. Miles, P.K. Sahota.* Children's Hospital, University of Missouri-Columbia, Columbia, MO.

Autism is a neurobehavioral disorder diagnosed by abnormal social, language and repetitive behaviors. Sleep disruption is an additional symptom that interferes with family functioning and may effect outcome. Because the nature and frequency of sleep problems in autism is poorly understood, we analyzed parental sleep reports for 163 children seen in the Autism Clinic at the University of Missouri from 1996-2002. The prevalence of insomnias (initiating and/or maintaining sleep), parasomnias (night wandering, sleepwalking, enuresis, bruxism, night terrors, etc.) and circadian rhythm disorders was determined and subject comparisons were made. We found a high prevalence (85.3%, 139/163) of any sleep disorder, regardless of age, IQ, seizures, regressive onset, dysmorphology or essential/complex designation. Essential autism (not dysmorphic, normal HC, structurally normal brain) is a more homogeneous, genetic autism subtype. Insomnia occurred in 69.3% (113/163) of subjects, with no difference in problems of sleep initiation alone (14.1%, 23/163) versus sleep maintenance alone (11.7%, 19/163). There was no significant difference in the prevalence of insomnia between the essential and complex groups. Parents reported a higher prevalence of parasomnias (77.3%, 126/163) than insomnias. The most common parasomnias were night wandering (20.9%), bruxism (24.5%), snoring (34.4%), and nocturnal enuresis (31.3%). All of these parasomnias were significantly more common than expected in typical children. Night time wandering occurred 2.4 times more in children with essential autism, suggesting that this may be a problem more specific to autism than complex developmental disorders. Night time enuresis was more frequent in the 5 - 11 year olds with complex autism ($p = .024$). Between the two broad categories of insomnia and parasomnia, subjects were more likely to suffer from parasomnias alone (26.4%, 43/163) than from insomnia alone (6.1%, 10/163), [$p = 7.3 \text{ e-}7$]. There were no significant findings of circadian rhythm disorders in this population. These results indicate that sleep problems, particularly the parasomnias, are common in autism and occur in all autism subgroups.

The neuropathology of Amish lethal microcephaly. *K.A. Strauss¹, R. Pfannl², D.H. Morton¹*. 1) Clinic for Special Children, Strasburg, PA; 2) Harvard Combined Program in Neuropathology.

Introduction: Amish lethal microcephaly (ALM) is a recessive disorder prevalent among Old Order Amish of Lancaster County, PA. The putative mutant protein is an inner mitochondrial membrane transporter which mediates deoxynucleotide diphosphate flux in vitro, and contains a structural motif predicting high-affinity binding to 2-ketoglutarate. Affected children have extreme micrencephaly, massive 2-ketoglutaric aciduria, and early death. We present neuropathology from a deceased 4 month old infant with ALM. Results: The brain weighed 91 grams. Frontal lobes were smooth and rudimentary, with diminutive anterior callosal and corticospinal fibers. Increasing convolution and lamination progressed occipitotemporally. Leptomeninges were focally thickened over the posterior cerebellum and anterolateral brain base; microscopically these were thick neuroglial heterotopias connected by bridge-like extensions to underlying neuropil. There were numerous heterotopic neurons in the intermediate zone within a dense background of reactive astrocytes and vacuolated neuropil. Symmetric, confined, and severe basal ganglia lesions were microscopically characterized by neuronal degeneration, mineral deposits, reactive astrocytosis, and new vessel formation. Conclusions: Enumeration of cells in the neocortical pseudostratified ventricular epithelium (PVE) is linked to histogenetic events that follow. Early cell cycle disruptions along the neuronogenetic gradient affect both spatial order and relative cell composition of the neocortical protomap; the abnormal pattern becomes amplified as histogenesis proceeds. Thus the most hypoplastic brain regions were also histologically most chaotic, remaining radially undifferentiated, with aberrant radial glia, patchy interruptions of the pial migration barrier, mixed heterotopias on the cortical surface, remnant subplate neurons in the intermediate zone, and a numerical dominance of astrocytes. Biochemical vulnerability of the PVE is based on its extraordinary proliferative pace, which demands synchronous nuclear and mitochondrial DNA replication, rapid protein accretion, and a sustained elevation of aerobic energy flux.

Intermediated Phenotype Between Amniotic Bands Sequence and Adams Oliver Syndrome. *F. Suarez, ML. Tamayo, J.C. Prieto.* Instituto de Genetica Humana, Universidad Javeriana, Bogota, Colombia.

Absence defects of limbs, scalp and skull, also know as Adams Oliver syndrome, is characterized by the presence of congenital aplasia cutis, variable terminal transverse defects in upper and lower extremities. The amniotic band sequence is a set of adhesions, mutilations and deformities, attributed to amniotic bands that disrupt fetal parts. Both entities, share clinical findings. In some patients with Adams Oliver syndrome had the initial diagnostic of amniotic band sequence. We report a Masculine patient, 26 months of age, product of first pregnancy, was born by cesarean delivery due to hydrocephaly. The mother was 26 years old, with nonsyndromic neurosensorial deafness of unknown cause, and the father was 25 years old with deafness secondary to meningitis. Physical examination showed turricephaly, shortened front to back diameter of the skull, small forehead, mid-face hypoplasia and linear right nostril defect. Accessory nipple in right hemithorax and a thoracic spine skin dimple. Cutaneous syndactyly, short fingers and terminal transverse defects with constricting rings in hands, short and small toe nails. He has no scalp or skin defects. Magnetic resonance of the brain showed fused thalamus, agenesis of the corpus callosum, obstructive hydrocephalus and skull malformations. Thoracic spine X ray showed bony defect of the posterior thoracic vertebral arches. Chromosomes were normal (46,XY). The physical findings of this patient suggest a secondary sequence to amniotic bands; nevertheless he presents other signs that have been described in the Adams Oliver Syndrome, including the terminal transverse defects in hands, accessory nipples, and short and partially syndactylous fingers and toes. In addition the patient presents with lobar holoprosencephaly, hydrocephaly, severe craniosynostosis and neural tube defect at thoracic level, alterations that are not characteristic of Adams Oliver syndrome, but are not either related of common way to the amniotic bands syndrome. This case shows that these two syndromes are part of a spectrum, which can share a common etiology in a vascular disruption sequence with an unknown genetic basis.

Correlation of genotype with the Autism Diagnostic Interview to stratifying cases of autism spectrum disorder.

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Autism spectrum disorders (ASDs) are neurodevelopmental disorders characterized by deficits in socialization and communication and, limited interests and preoccupations. Their prevalence is estimated to be 6/1,000 births. This study examined the relationship between the genotypes of candidate genes (e.g., *HOXA1*) and the phenotype of ASDs. We tested the hypothesis that outcome on subscores of the Autism Diagnostic Interview (ADI), a caregiver interview used to diagnose autism, would be predicted by the genotype of specific candidate genes. DNA was extracted from leukocytes isolated from peripheral blood. Each proband was genotyped for the candidate genes. ADI data was examined for probands with differing allelic variants. For example, summary scores evaluating communication indicated that probands with a AG or GG *HOXA1* genotype scored worse than probands with the AA genotype ($p < .001$). Analyses of individual communication items indicated that the G-allele predicted abnormal use of headshaking ($p < .015$) and pointing for communication ($p < .014$). Limited/repetitive interests were significantly more affected for AG probands than AA probands ($p < .001$). AG probands also showed evidence of problems at a significantly earlier age ($p < .002$) and spoke their first words significantly later ($p < .015$) than the AA probands. These data suggest that the G allele is associated with particular aspects of the autism phenotype, for example, nonverbal communication and obsessive interests, but not with deficits in other domains of ASDs such as reciprocal communication and social interaction. (Supported by NICHD RO124969 & PO125466, a Collaborative Program of Excellence in Autism.).

A novel congenital microcephaly phenotype: progressive infantile neuronopathy, cataracts, leukoencephalopathy and autonomic dysfunction. *K.J. Swoboda¹, A.F. Poss², K. Hyland³, J.M. Opitz²*. 1) Dept Neurol, Univ Utah Sch Med, Salt Lake City, UT; 2) Dept Human Genetics, Univ Utah Sch Med, Salt Lake City, UT; 3) Baylor Institute of Metabolic Disease, Dallas, TX.

We describe siblings with a novel neurodegenerative disorder characterized by: 1) congenital microcephaly with poor brain growth and progressive atrophy 2) paucity of central myelin, with a thin corpus callosum and progressive myelin loss 3) progressive neuromuscular weakness evolving in early infancy, most severely affecting the legs and ultimately leading to lower extremity paralysis, intercostal weakness, poor bulbar function, aspiration and tongue fasciculations and 4) autonomic dysregulation including ptosis, bowel dysmotility, gastroesophageal reflux, intermittent color changes, abnormal sweating and temperature regulation and apneic episodes. One sibling had congenital unilateral ptosis and cataracts, and died at one year of age with progressive respiratory failure. The second sibling developed recurrent generalized seizures by 4 months of age; cataracts did not become apparent until after 8 months of age. Parents are nonconsanguineous and have a healthy 5 year old son. Electromyography, muscle and nerve biopsy studies revealed a pattern of abnormalities similar to that seen in 5q SMA, but anterior horn cell drop out on autopsy could not be confirmed. Extensive metabolic evaluation excluded hexosaminidase deficiency, lysosomal disorders, serine deficiency, peroxisomal and mitochondrial disorders; high resolution karyotype and telomere FISH were normal. Cerebrospinal fluid analysis for neurotransmitter metabolites in one sibling revealed evidence of BH4 deficiency and decreased homovanillic acid consistent with a dopamine deficiency state. These abnormalities are almost certainly secondary. She had a partial response to treatment with BH4 and L-dopa/carbidopa with improved ptosis, alertness and circulatory instability, but continued to demonstrate progressive brain involvement and died of an ARDS-like illness at 14 months of age. Identification of similarly affected infants could help to further clarify the genetic and pathophysiologic basis of this disorder.

Congenital visceral and neurocutaneous glomangiomas, an aneurysm of the subhepatic inferior vena cava and diffuse arterial dysplasia. *D.S. Huff¹, P.J. Zhang³, J.A. Golden¹, D.M. McGinn-McDonald², E.H. Zackai².* 1) Dept Pathol, CHOP, Phila, PA; 2) Div of Human Genet and Mol Biol, CHOP, Phila, PA; 3) Pathol and Lab Med, University of Pennsylvania Medical Center, Phila, PA.

Introduction: Glomus bodies are normal intradermal thermal regulating arteriovenous anastomoses containing specialized perivascular smooth muscle cells called glomus cells. Glomus tumors, derived from glomus cells, are typically solitary, solid, perivascular cutaneous tumors of adults. Glomangiomas are a cystic variant of glomus tumors. Multiple glomus tumors or its variants (glomangiomas) are rare and may accompany neurofibromatosis. We present an infant boy with an unusual form of glomangiomas. **History:** The neonate first presented with coagulopathy and cerebral infarcts of intrauterine origin. He subsequently developed pulmonary hypertension, recurrent retinal lesions, a blue subcutaneous epigastric cyst and aneurysm of the subhepatic inferior vena cava with an arterial-vena caval shunt. He died at two months of age with heart failure and Kasabach-Merritt syndrome. **Autopsy:** The 10 x 6 x 5 cm aneurysm of the inferior vena cava filled the right abdomen from diaphragm to pelvis and harbored a 4 x 3 x 2 cm glomangioma in its wall. Multiple glomangiomas were also found in the renal hilum, thyroid, thymus, esophagus, cecum, hepatic hilum, leptomeninges, and skin of breast and epigastrium. Fibromuscular dysplasia involved systemic and pulmonary arteries. Patchy polymicrogyria and focal renal hypoplastic cystic dysplasia suggested midgestational vascular disruption sequences due to the vascular tumors and dysplasia. **Discussion:** Glomangiomas occur in children, may be congenital and is usually benign. Autosomal dominant forms are associated with defects of the Glomulin gene in the VMGLOM locus of 1p21-22. Most involve the skin or subcutaneous tissue of extremities. Rarely they involve venous walls and are associated with varicose veins. Our patient had an unusual form of congenital glomangiomas with extensive visceral involvement including the wall of the inferior vena cava which led to a fatal aortic-caval fistula and caval aneurysm.

High rate of non-penetrance in a six-generation family with long QT syndrome (LQT2) due to a HERG mutation. *C. Ohle¹, M. Qi³, J. Robinson², L. Ning³, Y. Xu³, C.J. Kim¹, A.J. Moss², T.E. Kelly¹.* 1) Division of Medical Genetics, University of Virginia, Charlottesville, VA; 2) Department of Medicine, University of Rochester, Rochester, NY; 3) Department of Pathology and Laboratory Medicine, University of Rochester, Rochester, NY.

The proband, an 18 year old female, was found to have long QT syndrome (LQTS) following successful resuscitation after cardiac arrest. Of the five genes now known to cause LQTS, the proband was found to have mutation in the HERG gene, G1128A/Q376sp. Subsequent study of the family included ECG and mutational analysis. Exon 5 of the HERG gene, the region covering the location of the possible mutation, was sequenced by dye terminator cycle-sequencing on an ABIprism DNA sequencer. This analysis yielded a six-generation kindred with numerous gene-positive, elderly individuals with no history of syncope or arrhythmias and a normal ECG. The proband's obligate heterozygous mother died at 38 years of age of cardiac arrest. A two year old, gene-positive son of the proband had LQTS by ECG. In this large family, the majority of individuals carrying the HERG mutation had no clinical symptoms of LQTS and a normal ECG. Factors which influence the penetrance of the phenotype of this mutation may be epigenetic or environmental, but none have been identified at present.

Management of aortic disease in the Marfan syndrome: a decision analysis. *R.E. Pyeritz, S.Y. Kim, N. Martin, E. Hsia, D.A. Albert.* Divisions of Medical Genetics and Rheumatology, Univ Pennsylvania Sch Med, Philadelphia, PA.

Marfan syndrome (MFS) is a common inherited defect in connective tissue caused by a mutation in the gene coding for fibrillin-1. The major cause of mortality is dissection of the ascending aorta, a complication that can be mitigated somewhat by medical therapy with β -adrenergic blockade and exercise modulation, and largely prevented by replacement of the aortic root with a prosthetic conduit. The standard surgical approach since the mid-1970s has involved a composite graft with a prosthetic aortic valve. Recently, approaches that spare the native aortic valve have gained currency, although the long-term effectiveness of the technique is unclear. We employed classic decision analysis to compare the efficacy of the 2 surgical therapies with each other and medical therapy. Using DATA Professional, we developed a Markov model decision analysis comparing the two surgical options with watchful waiting utilizing medical therapy. In the decision tree, 505 separate elements were assigned probabilities based on published statistics on outcomes and complications as well as on the natural history of aortic disease in Marfan syndrome. We then generated life-expectancy estimates for a hypothetical cohort of 30-year-old patients with Marfan syndrome and aortic dilatation. The results indicate that the composite graft option increases life-expectancy more than medical therapy (life-expectancy of 19.91 yr versus 19.50 yr from the age of 30), and both were preferred to surgery with a valve-sparing procedure (19.06yr). This analysis shows that, as expected, definitive repair of the aortic root with a composite graft leads to improved survival compared to medical therapy. However, in comparing the 2 surgical approaches in this relatively young population, the risk of serious adverse outcomes of anticoagulation, which is part of the management of the composite graft, is not as risky as re-operation, which is increased in the valve-sparing option. Sensitivity analysis will be conducted to examine specific subgroups such as those with a large aorta at presentation, those with a rapid increase in aortic diameter, and those with a family history of dissection.

DiGeorge Anomaly in the Absence of Deletion 22q11.2. *A.F. Rope, E.K. Schorry, R.J. Hopkin, H.M. Saal.* Division of Human Genetics, Cincinnati Children's Hospital Cincinnati, OH.

DiGeorge Anomaly (DGA) is defined as the presence of at least two of the following features: (1) cellular immune deficiency and/or absence of part or all of the thymus; (2) symptomatic hypocalcemia and/or parathyroid deficiency; (3) cardiovascular malformation (CVM). DGA is the most commonly seen as part of velo-cardio-facial syndrome (VCFS), resulting from an interstitial deletion of chromosome 22q11.2 and accounts for >90% of subjects with DGA. Records from individuals who were negative for deletion 22q11.2 studies (n = 308) were reviewed for the presence DGA. Dysmorphic facial features (n = 142), cognitive dysfunction (n = 103), CVMs (n = 96), velopharyngeal insufficiency (n = 66) and cleft palate or Robin sequence (n = 51) were the most common features present in this population. Fourteen cases of DGA in the absence of deletion 22q11.2 were identified. The two most commonly recognized etiologies were diabetic embryopathy and chromosome abnormalities (three cases of each). Also seen were one case each of cocaine embryopathy and CHARGE association. There were six cases of children with multiple anomalies where a specific diagnosis was not identified. In the spectrum of VCFS, the incidence of CVMs, hypocalcemia and immune deficiency is roughly 75%, 60% and <5% respectively. A large number of subjects with VCFS do not meet criteria for DGA and a significant portion of DGA is not accounted for by VCFS. As clinical geneticists it is important to use precise terminology, as it has implications for medical management and genetic counseling. As communicated by Wulfsberg et al in 1996, nomenclature for these entities has proven challenging. We propose that VCFS should be defined by the interstitial microdeletion at 22q11.2. Depending on the criteria outlined above, proper diagnostic terminologies would include DGA in the absence or presence of deletion 22q11.2 and VCFS with and without DGA. These designations continue to afford proper historical recognition to Dr. DiGeorge and refer to the most classic phenotype of deletion 22q11.2.

Searching for PTPN11 mutations in CFC and Costello syndrome patients. A.P. Peiffer¹, M.B. Bamshad¹, J.O. Opitz¹, D.V. Viskochil¹, M.L. Leppert², J.C. Carey¹. 1) Pediatrics, University of Utah, Salt Lake City, UT; 2) Human Genetics, University of Utah, Salt Lake City, UT.

Individuals with Noonan's syndrome have dysmorphic facial features, short stature and cardiac anomalies. A gene for Noonan's syndrome has recently been identified (Tartaglia *et al.* 2001 *Nat Genet* 29:465); PTPN11 encodes a nonreceptor protein tyrosine phosphatase, a component of intracellular signalling transduction pathways. Patients with cardiofaciocutaneous syndrome (CFC; OMIM #115150) or Costello syndrome (OMIM #218040) have clinical features similar to individuals with Noonan's syndrome but with additional facial, cardiac or cutaneous findings. Therefore it is possible that CFC and Costello syndrome are genetic variants of Noonan's syndrome. We collected phenotypic data and DNA samples from 31 patients with CFC or Costello syndrome. We have started searching for PTPN11 mutations in these individuals by direct sequencing. To date, we have screened exons 3, 7 and 8 reported to contain missense mutations in Noonan's syndrome and have found no PTPN11 mutations in our CFC or Costello patients. We are continuing to screen the remaining PTPN11 exons.

Cerebral and abdominal aortic aneurysms in a family with thoracic aortic aneurysms and dissections. *V.T. Tran, D.K. Lemuth, D.M. Milewicz.* Department of Internal Medicine, The University of Texas Medical School, Houston, TX.

Familial thoracic aortic aneurysms and dissections (TAAD) is an autosomal dominantly inherited condition with reduced penetrance and variable expression. Genes predisposing individuals to this condition have been mapped to 5q13-14 and 11q23. Abdominal aortic aneurysms (AAA) can be familial with most studies suggesting an autosomal recessive mode of inheritance. Cerebral aneurysms (CA) can also be inherited within families, although the inheritance pattern is less clearly established. We have identified a family in which 7 affected members spanning 3 generations have had dilatation or rupture of thoracic aortic aneurysms (TAAD), abdominal aortic aneurysms (AAA) or cerebral aneurysms (CA). In this family, two women were affected with cerebral aneurysms diagnosed at ages 52 and 55 years of age. Two women and one man were affected with abdominal aortic aneurysms or rupture at ages 53, 60 and 62 years of age, respectively. Two men were diagnosed with thoracic aortic aneurysms or dissections at ages 67 and 76 years of age. Segregation of the aneurysm phenotype is autosomal dominant with male-to-male transmission. No stigmata of Marfan syndrome or any other connective tissue disorder were present in the affected individuals. Described here are the clinical features of a novel, autosomal dominantly inherited syndrome involving TAAD, AAA and CA, with a variable age of onset and clinical presentation.

Visceral arteriovenous malformations, mucocutaneous telangiectasia and juvenile polyposis in a three-generation family. *G.M. Repetto¹, M. Linares², A. Peña³, F. Garay^{1, 2}, P. Harris¹, I. Concha².* 1) Dept Pediatrics, Hosp Univ Catolica de Chile, Santiago, Chile; 2) Hospital Padre Hurtado, Santiago, Chile; 3) Hospital Sotero del Rio, Santiago, Chile.

We report a family with pulmonary and hepatic arteriovenous malformations (AVM) and mucocutaneous telangiectasia fulfilling clinical criteria for hereditary hemorrhagic telangiectasia (HHT), in association with generalized juvenile polyposis (JP), affecting four individuals in three generations. The grandmother, now 45 years old, developed recurrent epistaxis in late childhood and has mucocutaneous telangiectasis. She was diagnosed with JP at age 15, requiring several polypectomies due to bleeding. The polyps did not show telangiectasis. Two of three offspring are also affected: a 20 year old daughter who has telangiectasis of skin and mucosae and has had several intestinal polypectomies starting at the age of 15 years, and a 10 year old son who has hepatic and pulmonary AVMs, mucocutaneous telangiectasis, JP and hypertrophic osteoarthropathy. A 25 year old daughter is healthy. The affected daughter has a 1 year old son with pulmonary AVMs, one of which required embolization due to hypoxemia. He has no evidence of intestinal polyps. Both boys have required embolization of pulmonary AV malformations due to hypoxemia. Brain MRIs in the adults and CTs in the children were normal, as well as imaging studies of the chest and abdomen in the adult females.

Other cases of a similar association have been published (MIM 175050), the first by Cox et al in 1980, suggesting that this is a distinct clinical entity, rather than coincidence of autosomal dominant JP and HHT. Further studies are necessary to elucidate whether this condition is caused by mutations in genes that cause isolated JP or HHT, has a different single gene etiology or could be explained as a contiguous gene syndrome.

A clinical and genetics study and 23 years follow up on hereditary hyperplasia gingival in a large family in Khorasan, North East of Iran. *K. Ghodsi¹, A. Banihashem², M. Tamizy², M. Golkhandan², R. Akbarzadah¹, M. Gorgi Nejad¹.* 1) Medical Genetics, Mashad University of Med Sch, Khorasan, Khorasan, Iran; 2) Dental School, Mashad University of Med Sch, Khorasan, Khorasan, Iran.

A clinical and genetics study and 23 years follow up on hereditary hyperplasia gingival in a large family in, North East of Iran. Kazem Ghodsi¹, Saeid Ali Banihashem², Mahmood Tamizy², , Reza Akbarzadeh¹, Mohsen Gorgi Nejad¹ and Mahmood Rahimi Golkhandan². Department of Medical Genetics¹, Ghaem Hospital, Faculty of Medicine, Dental school², Mashhad University of Medical Sciences Mashhad Iran. Hereditary hyperplasia gingival is a very rare genetically heterogeneous disorder. It is the most frequently reported to be transmitted as an autosomal dominant trait, but autosomal recessive inheritance has also been reported. The clinical presentation of hyperplasia gingival is variable both in the distribution and in the degree severity of expression. Here we reported a family with 13 affected individual in four generations living in Iran. The proband, the oldest man in family presented with a massive gingival overgrowth, at a regional dental clinic 23 years ago and was treated with extraction of all his teeth and gingival surgery to reduce the overgrowth. Recurrence was not observed. Other members of his family, (8 females, and 4 males) who were also affected have been referred for assessment and treatment. In all cases, the hyperplasia developed after the eruption of teeth and recurrence was observed after surgery in all patients who retained some teeth. Pedigree analysis suggests an autosomal dominant inheritance of hyperplasia gingival in this family. However, autosomal recessive inheritance cannot be ruled out as the family is from a small village in Khorasan, Iran, with possibility of relatedness between families. Additionally, although the proband and his affected children were apparently from a non-consanguineous marriage, other affected individuals are clearly from consanguineous mating. As expected karyotyping revealed no chromosomal abnormality. Further genetic analysis are underway.

Shortening of the bones of the hand in genetically characterized PHP-Ia patients. *L. de Sanctis*¹, *D. Romagnolo*¹, *M.R. Andreo*², *M. Olivero*³, *I. Dianzani*⁴, *C. de Sanctis*². 1) Osp Inf Regina Margherita, Univ Torino, Torino, Italy; 2) Div Pediatr Endocrinology, Osp Inf Regina Margherita, Torino, Italy; 3) IRCC, Candiolo, Univ Torino, Italy; 4) Dept Pediatr and Genet, Univ Piemonte Orientale, Italy.

Pseudohypoparathyroidism type Ia (PHP-Ia), caused by mutations in the *GNAS1* gene, is characterized by resistances to PTH, TSH, FSH/LH and Albright Hereditary Osteodystrophy (AHO), a heterogeneous phenotype described in different disorders. AHO comprises short stature, obesity, subcutaneous calcifications, mental retardation and metacarpophalangeal (MCP) shortness, the most specific sign. Data on MCP shortening in *GNAS1* characterized PHP-Ia patients have not been reported so far. To delineate a typical phenotype marker in PHP-Ia we evaluated the severity of MCP shortness in 11 *GNAS1* characterized patients and we searched for a correlation between MCP shortness and *GNAS1* mutations. Direct sequencing of the whole coding regions and exon/intron boundaries of the *GNAS1* gene allowed to identify the causal mutation in 11 PHP-Ia subjects followed in several Italian pediatric endocrinology units. Hand radiographs were collected from the 11 characterized patients and MCP shortening was evaluated using the method described by Poznanski et al., 1972. MCP shortness and predicted protein structure abnormalities for each identified mutation were considered. In the 11 PHP-Ia patients metacarpals (from 2,8 to 4,5 SD; IV-V above all) and distal phalanges (from -2,7 to 4,2 SD; I above all) were the most shortened bones. Patients with frameshift *GNAS1* mutations that predict for subversion of protein structure were not associated with more severe MCP shortening. One mutation found in various families and another in twins were not associated with similar MCP shortening. In conclusion, a genotype/phenotype correlation cannot be drawn between *GNAS1* mutations and bones shortening as a phenotype marker.

Chorea-acanthocytosis in a large French-Canadian kindred with dominant or pseudo-dominant inheritance and variable expressivity. *E. Andermann*^{1,2}, *A. Badhwar*^{1,2}, *A. Al-Asmi*¹, *C. Shustik*³, *F. Dubeau*¹, *S. Mercho*^{1,2}, *A. Sano*⁴, *F. Andermann*^{1,5}. 1) Dept. of Neurology and Neurosurgery, McGill Univ. Montreal, QC, Canada; 2) Dept. of Human Genetics; 3) Dept. of Internal Medicine, McGill Univ. Montreal, QC, Canada; 4) Dept. of Neuropsychiatry, Ehime Univ., Ehime, Japan; 5) Dept. of Pediatrics, McGill Univ. Montreal, QC, Canada.

We present a large family with neuroacanthocytosis (CHAC; OMIM-200150), familial temporal lobe epilepsy and unusual inheritance. CHAC is an autosomal recessive, rarely dominant, neuro-degenerative disorder with gradual onset of hyperkinetic movements and acanthocytosis. Features include chorea, orofaciolingual dyskinesia, tics, dysarthria, dysphagia, areflexia, seizures and dementia. The gene is located on chromosome 9q21 (Rampoldi et al, 2001). 3 siblings and their maternal first cousin from a consanguineous marriage presented with familial temporal lobe epilepsy and later developed involuntary movements and dysphagia. All 4 patients had evidence for manic-depressive disorder and cognitive deterioration. Carbamazepine and lamotrigine worsened involuntary movements. MRI showed caudate atrophy and increased iron deposition in the globus pallidus bilaterally. EEG and video-telemetry confirmed ictal and interictal temporal abnormalities. DNA testing (Huntington, oculopharyngeal muscular dystrophy) was negative. Blood smears revealed acanthocytosis. The family originates from the Saguenay-Lac St. Jean region of Quebec. Thirty individuals in 4 generations had varying combinations of tics, chorea, epilepsy, psychosis, ptosis and dysphagia. Genetic linkage and mutation studies are underway.

In conclusion patients presented with familial temporal lobe epilepsy, unusual for CHAC. Coexistence of choreiform movements, mood disorder, mental deterioration and acanthocytosis led to the diagnosis. Inheritance appears to be autosomal dominant, but may be pseudo-dominant due to founder effect in this population.

Patients with Multiple/Plexiform Schwannomatosis: Distinct Clinical Entities or NF-Variants? *L.L. Baumbach¹, E. Estrella¹, M. Bauer²*. 1) Univ Miami Sch Medicine, Miami, FL; 2) Miami Children's Hospital, Miami, FL.

The neurofibromatoses (NF) represent a clinically heterogenous collection of disorders that share certain common clinical manifestations, while underlying molecular and pathological mechanism(s) responsible for diverse disease manifestations remain unclear. Appreciation has arisen for variant NF forms. One of the most intriguing is multiple schwannomatosis (MSM), which occurs without any clinical evidence of NF-2. It is still controversial whether MSM represents a third form of NF, or a mosaic form of NF-2. We report our clinical investigations of four unrelated patients who present with either MSM or Plexiform Schwannomas (PSM). The patients have been otherwise healthy, and have had extensive clinical and radiological evaluations that have failed to detect any other features consistent with NF-2. Family history is negative for any clinical features or symptoms consistent with NF for each of the probands. Patient #1, a 19 yr-old Caucasian male, has multiple spinal schwannomas, including a hemorrhagic schwannoma spanning L-4 to S-1, which was surgically removed. Patient #2, a Hispanic 27 year old female, has a long history of peripheral nerve schwannomas. Patient #3, a 20 yr.-old Caucasian male, has a chronic history of a thoracic spinal plexiform neurofibroma/ schwannoma. Patient #4 is a 3 yr.-old girl with multiple schwannomas involving the trigeminal nerves, a large plexiform neurofibroma in the left masticator space, and vagus nerve schwannomas in the left jugular carotid sheath. These patients share in common apparently *de novo* events, childhood onset of symptoms, and confinement of symptoms to benign schwannomas/plexiform neurofibromas involving the CNS or PNS. We are striving to complete *NF2* mutation analysis in peripheral blood and affected tissue specimens from these patients. Continued clinical and molecular investigation of such rare patients will provide important new insights into the etiology of variant presentations of NF-1 and NF-2.

Life threatening events and severe manifestations of Prader Willi syndrome in infancy. *Y.S. Choy¹, S.K. Tan², SK. Tan⁵, M.Z. Norzila³, L.C. Hung⁴.* 1) Genetics Unit, Pediatrics Institute, Kuala Lumpur Hospital, Kuala Lumpur, Malaysia; 2) Cytogenetic Department, Kuala Lumpur Hospital, Kuala Lumpur; 3) Respiratory Unit, Pediatrics Institute, Kuala Lumpur Hospital, Kuala Lumpur; 4) Cardiology Unit, Pediatrics Institute, Kuala Lumpur Hospital, Kuala Lumpur; 5) Division of Molecular Pathology, Institute of Medical Research, Kuala Lumpur, Malaysia.

Infantile failure to thrive and hypotonia with developmental delay and obesity later in life is a typical natural course of many individuals with Prader Willi syndrome described in the literature. With the increase in awareness of the condition, more patients with Prader willi syndrome are now being diagnosed in the neonatal period. 18 patients were confirmed having Prader Willi syndrome over the past two years in the Pediatrics Institute, Kuala Lumpur Hospital by methylation PCR. 12 of the cases (67%) were deletion positive using SNRPN probe. 13 of them were diagnosed in infancy and 9 of them (50%) were diagnosed in neonatal period because of severe central hypotonia associated with respiratory distress and feeding difficulty. 12 of them have more than twice admissions to the hospital for acute life threatening events during infancy. Majority of them were due to pneumonias. Two of the patients required gastrostomy feeding and two needed temporary nasogastric tube feeding. 4 of them had cardiomyopathy and 2 of them had pericardial effusion and cardiac tamponade. Two of the patient with cardiac involvement also had hypothyroidism and the treatment of which resulted in clinical improvement. Two of the patients succumbed to the life threatening events and one suffered severe hypoxic ischemic damage of the brain. Six patients had obstructive sleep apnoea and oxygen desaturation during sleep. Home oxygen therapy and vigorous airway management in these patients resulted in general clinical improvement and prevention of hospitalization.

Central Nervous System Anomalies in Seckel Syndrome: Report of a Case with Central Apnea and Acquired Chiari Malformation. *D.E. Halbert, G.S. Pai, J.K. Cure.* Pediatrics, Medical Univ of South Carolina, Charleston, SC.

Seckel syndrome, also known as Bird-Headed Dwarfism, is a rare syndrome which consists of low birth weight, dwarfism, microcephaly, large eyes, beaklike nose, narrow face, and receding lower jaw. Associated cranial and CNS anomalies have been better elucidated in recent years.

Here we describe a patient with severe microcephaly and an acquired Chiari malformation. The patient was born at 38 weeks gestation to a 20 year old African American Mother. Family history was negative for mental retardation, microcephaly, or consanguinity. Subsequent cytogenetic analysis at 550 band resolution revealed normal XYkaryotype.

The patient suffered from developmental delay and central apnea. MRI study of the brain at 3 months of age demonstrated a Chiari type I malformation with the cerebellar tonsils lying at the lower mid C3 cervical spine level. A prior high resolution MRI using 3 millimeter slices was performed at 1 week of age did not demonstrated the Chiari malformation. The new finding of the malformation was surprising since Chiari malformations generally thought to be congenital anomalies. Subsequent CT scan of the skull with 3 dimensional reconstruction demonstrated bilateral lambdoid and sagittal synostosis.

The patient underwent decompression of the Chiari malformation and cranial reconstruction. Unfortunately, the patient was not able to be extubated due to continued apnea which was felt to be centrally mediated. A tracheostomy was placed but the patient was not able to be weaned from mechanical ventilation. The child died at 4 months of age due to sepsis. An autopsy was not performed.

This case adds to the clinical description of children with Seckel syndrome. It also demonstrates the development of a Chiari malformation postnatally.

Clinical and genetic characterization of familial juvenile hyperuricemic nephropathy. *T.C. Hart^{1,2}, M.M. Gorry¹, H. Zhu¹, M.M. Barmada², A.J. Bleyer³.* 1) Dept Oral Med/Pathology, Univ Pittsburgh, Pittsburgh, PA; 2) Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA; 3) Dept Nephrology, Wake Forest Univ, Winston Salem, NC.

FJHN is an autosomal dominantly transmitted kidney disease that results in precocious gout and kidney failure. Clinical findings vary greatly, and as the disease causing gene is unknown, clinical characterization of FJHN is poor, primarily because genetically affected individuals with mild expression can not be reliably identified. The purpose of this study was use linkage analyses to identify genetically affected individuals and characterize clinical findings in these individuals. Genetic linkage studies performed on 62 individuals from a family segregating FKHN localized the disease gene to a 2 Mb region of chromosome 16p (ZMAX = 12.5 @ D16S3041, theta= 0.01). Haplotype analysis permitted identification of family members who inherited the FJHN disease allele. Linkage and haplotype studies permitted identification of 25 unaffected family members and 37 genetically affected individuals. 83% (31/37) of genetically affected family members suffered from hyperuricemia and either renal failure or a reduced fractional excretion of uric acid. Six females who inherited the FJHN disease haplotype had normal serum uric acid levels despite low fractional excretion of uric acid in four of them. There was considerable overlap between serum uric acid values and fractional excretion of uric acid in genetically affected and unaffected individuals. Only 38 % of genetically affected individuals suffered from gout. Conclusions: using genetic linkage studies we sublocalized the FJHN gene to chromosome 16p13 and to determine which family members were genetic carriers of the FJHN disease gene. While most patients with FJHN suffer from hyperuricemia, reduced fractional excretion of uric acid, and renal failure, not all characteristics are fully penetrant. The variable clinical expression of FJHN is described. These findings suggest that the clinical findings of FJGN vary from severe (lethal without renal transplant) to very mild, and that the definitive diagnosis of disease will likely require genetic testing.

A Novel Ehlers-Danlos-Like Syndrome with Vascular abnormalities and Tortuous Systemic Arteries: Linkage Analysis. *M.F. Ul Haque*¹, *A. Abdul Wahab*², *I.H. Janahi*², *S.H.E. Zaidi*¹, *A.S. Teebi*¹, *L.C. Tsui*¹. 1) Dept Genetics, Hosp Sick Children, Toronto, ON, Canada; 2) Dept Pediatrics, Hamad Medical Corp, Doha, Qatar.

The Ehlers-Danlos syndrome (EDS) is a heterogenous group of heritable connective tissue disorders characterized by skin hyperextensibility, joint hypermobility, and tissue fragility. Thirty two affected individuals from a large consanguineous kindred from Qatar were identified to exhibit similar phenotypic anomalies including skin hyperextensibility of variable degree without bruising and/or echymosis, hypermobility of small and large joints, and dilatation, tortuosity and stenosis of systemic, pulmonary, and coronary arteries. The facial features are peculiar and include epicanthic folds, saggy flat cheeks, an elongated face and micrognathia. Hernia is common and includes inguinal, diaphragmatic and/or hiatus hernia. Skin biopsies from affected individuals show normal collagen fibrils and elastin fibers. Although similar to the EDS phenotype, clinical spectrum of anomalies seen in this family do not fit with any previously described forms of EDS. From our analysis of the kindred, the affected individuals appear to be segregating a recessively inherited mutation. Linkage analysis is being conducted to determine whether the mutant locus in this family corresponded to any of the major loci previously implicated in both recessively or dominantly inherited forms of EDS. Intragenic or closely linked, flanking microsatellite markers are used. Assuming autosomal recessive inheritance there is no evidence for genetic linkage to any of the previously implicated loci for EDS. Further, affected individuals have also been excluded from cutis laxa, Ehlers-Danlos and Marfan syndromes, as well as several other mutations in the genes known to produce vascular abnormalities. We speculate that loss of function in a previously unidentified mutant gene is responsible for conferring this trait. A genome wide screening is currently underway to identify the region harboring this abnormal gene.

Ultrasound analysis of orbicularis oris muscle defects in relatives of individuals with cleft lip with or without cleft palate and their relatives. *S.M. Weinberg¹, K. Neiswanger¹, R.A. Martin², M.P. Mooney¹, R.S. Faix³, B.S. Maher¹, S.S. Petiprin¹, K.M. Bardi¹, R.F. Giles¹, A. Bowen³, M.L. Marazita¹.* 1) Sch Dental Med, Univ Pittsburgh, Pittsburgh, PA; 2) St Louis Children's Hospital, St Louis, MO; 3) Childrens Hospital, Pittsburgh, PA.

Oral-facial clefts affect between 1 in 500 to 1000 births worldwide. Like many other complex phenotypes, the search for susceptibility loci has had limited success. Recently, however, a sub-clinical defect of the orbicularis oris (OO) muscle, was detected through ultrasound in the first-degree relatives of children with CL/P. It has been hypothesized that OO defects may represent an alternative phenotype for gene mapping in families with CL/P. Herein, we present an analysis of OO muscle defects in unaffected relatives of CL/P probands ascertained as part of a large multiplex family study on the cleft phenotype and genotype. Ultrasound images were collected from unaffected relatives (n=69) and scored quantitatively and qualitatively by multiple raters for OO muscle defects. Measures from unaffected relatives were compared to those of 52 previously published controls. OO muscle defects were present in 52.3% of the relatives of CL/P probands versus 13.5% of controls ($\chi^2 = 19.07$, $p < .0001$). These results support the hypothesis that OO muscle defects are a subclinical indicator of increased susceptibility to CL/P. In addition, OO muscle defects may provide an alternative phenotype for mapping genes underlying CL/P. NIH grants DE13076 and RR00084.

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New Case of Verloes Syndrome. *JC. Prieto^{1,2}, C. Gutierrez¹*. 1) Inst de Genetica Humana, Univ Javeriana, Bogota, Colombia; 2) Hospital la Victoria, Departamento de Genetica, Bogota, Colombia.

We report the first case of Verloes Syndrome in one male in Colombia and the third one worldwide. Verloes et al. in 1990 describe two brothers that shared the following clinical characteristics: postnatal growth deficiency, obesity, coarse facial features with deep-set small eyes, and severe genital anomalies resulting in sexual ambiguity, including persistent müllerian structure. Mental retardation was profound in one; the other sib was only slightly retarded. In this report we describe a slightly mentally retarded post - puberal male. He had 19 years old and he had 46,XY chromosome constitution. Clinical features include moderated gynecoid obesity, coarse face, micropenis, small testis, persistence of Müllerian structures, and low gonadotrophin levels. Those anomalies do fit a previously reported syndrome by Verloes et al, although the general aspect of the propositus clearly resembles Borjeson-Forsman-Lehmann syndrome. In this new case the parents are no consanguineous and no other member of the family was affected, thus in this case we don't have evidence of autosomal or X-linked inheritance.

Syndrome of choanal atresia, ear anomalies, deafness and athelia associated with chronic interstitial lung disease.
D.K. Grange, S.C. Sweet, F.S. Cole. Department of Pediatrics, Washington University School of Medicine and St. Louis Childrens Hospital, St. Louis, MO.

We report a 12 month old girl with bilateral choanal atresia, ear anomalies, sensorineural hearing loss and athelia. She was born at 38 wks gestation to a 24 yr old G1 mother following a normal pregnancy with no teratogen exposures. She has sparse hair, small ears with skin tags on the superior helices, a preauricular pit, small nose with narrow alae nasi and a unilateral branchial cleft sinus tract. There is total absence of the nipples. Heart is normal by echocardiogram. Chromosome analysis was 46,XX. Despite surgical correction of choanal atresia, the patient has required continuous oxygen supplementation. At 6 months, CT scan showed severe diffuse interstitial infiltrates and tiny cysts scattered throughout the lungs. Open lung biopsy revealed chronic interstitial pneumonitis with widening of the alveolar septae by an inflammatory infiltrate. Air spaces were partially obliterated by fibrous connective tissue. She has clubbing of the digits. Postnatal growth has been poor and she is below the 3rd percentile for all parameters. Thyroid function tests were normal. The features in our patient overlap with several previously described conditions, including methimazole embryopathy (choanal atresia, athelia, hypothyroidism), Bamforth syndrome (choanal atresia, cleft palate, abnormal hair, hypothyroidism) and ANOTHER syndrome (athelia, ectodermal dysplasia, hypothyroidism). The association of athelia, choanal atresia and renal tubular dysplasia was reported in 3 brothers by Hisama (AJMG, 1998). Two siblings with anomalies very similar to our patient were reported by Al-Gazali (Clin Dysmorphol, 2002); one had a small thyroid with normal function and interstitial fibrosis of the lungs on CT scan, and the other had hypothyroidism, chronic hypoxemia and died of pneumonia. Bamforth syndrome is caused by mutations in the gene for thyroid transcription factor 2 (TTF-2). TTF-1 is a related protein involved with thyroid specific gene transcription as well as pulmonary production of surfactant, and could therefore be a candidate gene for the disorder in our patient and those of Al-Gazali.

ATYPICAL FAMILIES FOUND WHILE SEARCHING FOR USHER SYNDROME IN COLOMBIA. C.P.

Gonzalez¹, D. Medina², N. Gelvez¹, M. Tamayo¹. 1) Instituto de Genetica Humana, Facultad de Medicina. Universidad Javeriana, Bogota, DC, Colombia; 2) Fundacion Oftalmologica Nacional, Bogota,D.C., Colombia.

Usher Syndrome (USH) is comprised of a group of autosomal recessive disorders characterized by congenital sensorineural hearing loss, vestibular dysfunction, and progressive Retinitis Pigmentosa (RP). This entity is clinically and genetically heterogeneous. The syndrome has been divided into three clinical types, based on the severity of hearing loss, the extent of vestibular involvement, and age at onset and progression of hearing loss. USH is the foremost cause of combined blind/deafness in the world. The prevalence in Colombia is estimated in 3.2/100,000 inhabitants. Our group has been working since 1984 in a screening program for USH, carrying out otological, ophthalmological, genetic studies and also, genetic counselling in affected individuals and their families. In this process, we found out that many patients that were given an initial Usher Syndrome diagnosis by the physician, after considering all the clinical and family traits together, were not the typical Usher Syndrome. We describe the cases and clinical traits of 3 atypical Usher Syndrome families. Family 1, showed RP and sensorineural deafness but in an autosomal dominant pattern in three generations. In Family 2, two of the affected presented autosomal recessive RP with sensorineural profound deafness, but two other affected siblings had only RP. The propositus of these families were diagnosed as Usher Syndrome when considered isolated. Family 3, product of a consanguineous marriage, showed RP in two women but one of them referred hearing loss, with three brothers with deafness and another one with possible RP. Our purpose is to emphasize that researchers and clinicians must be aware of certain cases that seems to be Usher Syndrome families, really are not. There is a high frequency of RP with sensorineural deafness in families without being Usher Syndrome.

Trigonocephaly-associated hypotelorism in an infant with trisomy 9q(p13@qter). *J.R. Corona-Rivera^{1,2}, A. Corona-Rivera¹, J.A. González-Ramírez², S. González-Abarca², T.A. García-Cobian¹, L. Bobadilla-Morales¹, E. Corona-Rivera¹.* 1) Laboratorio de Genética Humana, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jalisco, México; 2) División de Pediatría, O.P.D. Hospital Civil de Guadalajara Dr. Juan I. Menchaca.

Trisomy of the distal half of 9q appears to be responsible for the trisomy 9q syndrome phenotype. We describe an infant showing association of trigonocephaly and trisomy 9q. The proposita was born spontaneously to a 37-year-old, G3P2A0 mother after normal gestation and vaginal delivery, complicated with neonatal hypoxia. Aged 1 month showed: height 53 cm (-0.1 SD), weight 2400 g (-4.4 SD), head circumference 32 cm (-3.6 SD), facial asymmetry, wide sagittal suture and fontanel, trigonocephalic head from bulging of the forehead along the metopic suture, prominent eyeballs, hypotelorism, bulbous nose, long filtrum, thin upper lip, short neck, heart murmur, umbilical herniae, pilonidal dimple, clenched hands, and rocker-bottom feet. Apneic episodes and generalized tonic seizures were also observed. Echocardiogram exhibited tetralogy of Fallot. Cranial 3D CT scan confirmed trigonocephaly, hypotelorism and metopic and coronal synostosis. Intracranial structures were normal on CT scan as well as an abdominal ultrasonography. Peripheral blood lymphocytes karyotype showed a 47,XX,+del(9)(p13) in 65 metaphases analyzed after CTG-banding. The proposita died at two months of age. Clinical features in our case were as those observed in 9q trisomy for different segments, as well as complete trisomy 9, including early death. Patients with 9q trisomy were informed with microdolichocephaly and prominent forehead, besides other facial, spine, hand/foot, cardiovascular and urogenital anomalies. To our knowledge, this is the first instance of trigonocephaly-associated hypotelorism in a trisomy 9q therefore suggesting that craniofacial synostotic anomalies might be associated with 9p13@qter trisomy.

The face of Joubert syndrome: a study in dysmorphology and anthropometry. *S.R. Braddock, K.M. Henley, B.L. Maria.* Department of Child Health, University of Missouri, Columbia, MO.

Joubert syndrome is a recessive neurogenetic disorder characterized by hypoplasia of the cerebellar vermis, hypotonia, developmental delay, and abnormal breathing and eye movements. Specific neuroradiologic findings of the “molar tooth” sign aid the diagnosis. Associated phenotypic features described include facial, renal, limb and gastrointestinal malformations. However, a large cohort has not been systematically examined by a dysmorphologist. The purpose of this study is to document the dysmorphology and anthropometry of Joubert syndrome in a large population of affected individuals and to determine whether a specific pattern of malformation exists and is altered significantly with age.

Since its first description in 1969, much attention has been focused on the classic hindbrain malformation. This is characterized by hypogenesis and clefting of the cerebellar vermis with enlargement of the fourth ventricle, deepening of the interpeduncular fossa, and a lack of decussation of the thickened superior cerebellar peduncles, central pontine, and corticospinal tracts. To date there have been few reports and documentation of the physical phenotype of Joubert syndrome other than the neuropathology and behavior, though some 200 cases have been published. In 1999, Maria et al. described clinical features in 19 individuals with the condition. Their findings included a “typical” facies with high rounded eyebrows, broad nasal bridge, epicanthal folds, anteverted nares, a triangular shaped mouth with the tongue almost protruding and low set ears in addition to the vermis hypoplasia, hypotonia, developmental delay, and abnormal eye and breathing patterns. Other conditions sharing multiple features with Joubert syndrome include but are not limited to Ritscher-Schinzel (3C), Oral-Facial-Digital (OFD), Arima, Senior-Löken, Dekaban, and COACH syndromes.

We will present data from dysmorphology evaluations of 22 individuals, ages 14 months to 18 years, with Joubert syndrome. Anthropometric examinations will document whether the facies have distinguishing characteristics or represent the generalized effects of hypotonia.

Karyotype/phenotype correlations in duplication 4q: evidence for a critical region within 4q27-31 for preaxial defects. A. Battaglia¹, L. Hudgins³, S. Morelli², JC. Palumbos², JC. Carey². 1) Inst Child Neurology & Psych, Stella Maris nst/Univ Pisa, Pisa, Italy; 2) Division of Medical Genetics, Dept. of Pediatrics, University of Utah, SLC, UT; 3) Dept. of Pediatrics, Stanford University, CA.

Varying degrees of partial duplication of 4q (dup 4q) have been reported in over 70 patients since 1972. The majority of the cases include partial trisomy with breakpoints at q25 to ter, most due to unbalanced inherited translocations. The different breakpoints, the associated monosomies, and the varying ages of reported patients make it difficult to delineate a recognizable syndrome. However, there are five patients reported with preaxial defects, ranging from thumb hypoplasia to duplication, all having as well renal malformations. From this, Zollino et al. (1995) suggested the presence of genes involved in the development of the acrorenal field on 4q at 22-23. We describe 2 patients with dup 4q27 to ter, one of which is especially helpful in elucidating the critical region for thumb defects in dup4q. The first is a girl with developmental delay, microcephaly, growth delay, absent thumb/hypoplastic thumb and single kidney. Her karyotype is 46,XX, der(11)t(4;11)(q27;q24.2)mat as her mother carries a balanced 4;11 translocation. Thus she has partial dup 4q and deletion of distal 11q. The second patient has multiple anomalies, including double outlet right ventricle, and 46,XY,der(10)t(4;10)(q27;q26.3)pat as his father carries a 4;10 translocation. He has partial dup4q and deletion of distal 10q. He has normal thumbs, demonstrating the variability of this finding in dup4q. Analysis of the dup4q in the previously described patients with thumb defects and our patient indicates that the region of overlap is 4q27-31.3, which is more distal than originally suggested by Zollino et al. (1995). Of note, all 6 patients have renal malformations, consistent with their hypothesis that there are genes important in acrorenal field development in 4q27-31.

Neurocognitive Profile of Adults with 22q11.2 Deletion Syndrome. *E. Chow*^{1,2}, *M. Watson*², *D. Young*³, *R. Weksberg*^{4,5}, *A.S. Bassett*^{1,2}. 1) Department of Psychiatry, University of Toronto, Toronto, ON, Canada; 2) Clinical Genetics Research Program, CAMH, Toronto, ON, Canada; 3) Schizophrenia & Continuing Care, CAMH, Toronto, ON, Canada; 4) Department of Paediatrics, University of Toronto, Toronto, ON, Canada; 5) Clinical and Metabolic Genetics, HSC, Toronto, ON, Canada.

Background: 22q11.2 Deletion Syndrome (22qDS) has been associated with neurocognitive weaknesses in children. The neurocognitive profile of 22qDS adults is less well understood. **Objective:** To determine the neurocognitive profile of adults with 22qDS. **Method:** 45 adults with 22qDS were assessed using a comprehensive battery of neurocognitive tests. The 25 subjects with schizophrenia (22qDS-SZ) were compared with 20 subjects with no psychosis (22qDS-NP). **Results:** The 22qDS-SZ and 22qDS-NP groups had similar mean overall IQ (71.5 SD 7.7 vs. 73.3 SD 9.0, respectively), mean verbal IQ (71.9 SD 4.8 vs. 74.1 SD 8.5), and mean performance IQ (71.2 SD 5.1 vs 74.2 SD 10.2). The groups also had similar academic achievement in reading (Grade 6-7 level), spelling (Grade 6-7), and arithmetic (Grade 4), and mild to moderate impairment in attention, visual-spatial skills, motor and executive functioning. Compared to the 22qDS-NP subjects, 22qDS-SZ subjects had poorer performance in verbal learning (Rey Auditory Verbal Learning Test, $p=0.006$), delayed verbal recall (Wechsler Memory Scale-Revised, Logical Memory II, $p=0.01$) and executive functioning (Trail B, $p=0.004$). **Conclusion:** These findings in 22qDS adults are mostly similar to those reported in 22qDS children. There are significant differences in neurocognitive domains between psychotic and non-psychotic 22qDS subjects, consistent with the profile for other forms of schizophrenia.

Congenital Onychodysplasia and Juvenile Hypertrophy of Breast: A new syndrome. *T.C. Falik-Zaccai¹, M. Khayat¹, J. Govrin-Yehudain², L. Kogan², S. Linn³.* 1) Div Medical Genetics, Western Galilee Hosp, Nahariya, Israel; 2) Plastic surgery unit, Western Galilee Hosp, Nahariya; 3) Epidemiology unit, Rambam Medical Cent. Bruce Rappaport Faculty of Medicine, Technion, Haifa, Israel.

Familial Juvenile hypertrophy of the breast (JHB) is a very rare condition described only once in the literature. The pathology is limited to the breast, leading to gigantomastia in peripubertal females with otherwise normal growth and development. Familial Onychodysplasia and dysplasia of distal phalanges (ODP) is a mild bone dysplasia with normal height and no functional limitation. This is a very rare congenital syndrome that was also described only once before in the literature. We report here for the first time on a familial pattern of JHB accompanied by ODP. These two traits segregated in a four-generation family in an autosomal dominant (AD) fashion with complete penetrance among females (JHB and ODP) and partial penetrance among males (ODP only). There were no affected individuals in generations II and I, six affected individuals out of fourteen in generation III and six affected individuals out of ten in generation IV. One obligate carrier in generation II was asymptomatic. Five affected females had breast reduction surgeries and suffered curtailment of their social activity and cessation of schooling. The mothers of these five patients had normal breasts and nails while their fathers had ODP but no JHB. Eight affected males exhibit ODP with no JHB. Females that did not present with JHB had normal hands and feet. Thus, suggesting male to male transmission of the ODP and male to female transmission of ODP combined with JHB. Statistical analysis supported a possible cryptic chromosomal aberration or a common gene mutation responsible for the two traits. A germline new mutation or chromosomal aberration has possibly occurred in generation II leading to multiple affected in generation III; alternatively, a silent mutation exhibiting the anticipation phenomenon is the cause for the unusual phenotype. The genetic basis for this new syndrome is yet to be determined. The large kindred provides the basis for mapping and cloning of the possible gene responsible for this new syndrome.

Interstitial deletion del(9)(q22.32q33.2) associated with additional familial translocation t(9;17)(q34.11;p11.2) in a patient with Gorlin-Goltz syndrome and features of nail-patella syndrome. A.T. Midro¹, B. Panasiuk¹, Z. Tumer⁶, P. Stankiewicz⁷, J.R. Lupski^{7,8}, Z. Zemanova⁹, J. Brezinova⁹, B. Stasiewicz-Jarocka¹, E. Hubert², E. Tarasow³, W. Famulski⁴, E. Wasilewska⁵, K. Michalova⁹, N. Tommerup⁶. 1) Department of Clinical Genetics; 2) Clinic of Maxilla-Facial Surgery; 3) Department of Radiology; 4) Department of Clinical Pathology; 5) Clinic of Hematology, Medical Academy of Bialystok, Bialystok, Poland; 6) Institute of Medical Biochemistry and Genetics, Department of Medical Genetics, The Panum Institute, University of Copenhagen, Denmark; 7) Department of Molecular & Human Genetics; 8) Department of Pediatrics, Baylor College of Medicine, Houston, TX; 9) 3rd Medical Clinic, ILF UK, Praha, Czech Republic.

The phenotype of Gorlin-Goltz syndrome (NBCSS, 109400 OMIM), a Mendelian entity due to changes in the *PTCH*, has been reported in association with interstitial chromosomal 9q deletion. We present an 11-year-old girl with clinical features consistent with NBCSS including: bridging of the sella turcica, frontal and biparietal bossing, downward slanting palpebral fissures, mandibular prognathism, pectus excavatum, thumb abnormalities, spina bifida occulta at L5-S4, and basal cell nevi. Cytogenetic analysis using high-resolution banding techniques and fluorescence *in situ* hybridization (FISH) revealed interstitial chromosomal deletion del(9)(q22.32q33.2) involving the *PTCH* as a secondary breakage event to chromosome translocation t(9;17)(q34.1;p11.2)mat. Further FISH studies showed the localization of the translocation breakpoint on 9q34.11, between the BAC clone RP11-88G17 and *LMX1B*. The latter gene encodes a transcription factor, in which loss of function mutations are responsible for the nail-patella syndrome (NPS). Interestingly, some features in our proband (e.g. bilateral dysplasia of patella and abnormal shape of clavicle) and in her healthy sister, carrier of the same translocation, are also found in the patients with NPS. The chromosome 17p11.2 breakpoint was identified to map in the Smith-Magenis syndrome common deletion region, within two overlapping BAC clones CTD-2354J3 and RP11-311F12.

Immunodeficiency in Kabuki syndrome. *J.E. Ming, K.L. Russell, D.M. McDonald-McGinn, E.H. Zackai.* Division of Human Genetics, Department of Pediatrics, The Children's Hospital of Philadelphia and the University of Pennsylvania School of Medicine, Philadelphia, PA.

Immunodeficiency is associated with a number of genetic syndromes. Frequent infections, especially sinopulmonary infections, often occur in patients with Kabuki syndrome. For most patients, the possibility of an underlying immune deficit has not been investigated. A small number of individuals with Kabuki syndrome have been described with immune defects or autoimmune disease. We describe five patients with Kabuki syndrome evaluated at The Childrens Hospital of Philadelphia who have an immunologic abnormality and/or presented with an autoimmune condition. Patient #1 had a history of frequent episodes of otitis media. At 13 years of age, he presented with thrombocytopenia and hemolytic anemia. He was later hospitalized with Pneumococcal pneumonia. He had undetectable IgG levels, and decreased IgM and IgA levels. Patient #2 had recurrent otitis media requiring three sets of ear tubes and a hospitalization for an RSV infection. She subsequently presented with thrombocytopenia. She had low IgG and IgA levels and a low normal IgM level. Patient #3 had pneumonia in infancy and chronic otitis media. He had a decreased IgA level with low normal IgG and IgM levels. Patient #4 had three episodes of pneumonia and recurrent otitis media. He was noted to have low IgA and IgG3 levels with low normal levels of IgM and other IgG subclasses. Patient #5 presented with thrombocytopenia and anemia. He has a history of recurrent otitis media and *C. difficile* infection. His IgA level was borderline normal and his IgG level was in the low normal range. The relatively high frequency of antibody deficiency suggests that immunologic abnormalities are a manifestation of Kabuki syndrome. The immune defect resembles common variable immune deficiency. The autoimmune disease is likely to be a reflection of the underlying dysfunction of the humoral immune system. We recommend that immunologic testing focusing on the humoral immune system be performed at regular intervals.

A novel Treacher Collins-like syndrome in an Alaskan Inupiat community with autosomal recessive inheritance.

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Classic mandibulofacial dysostosis or Treacher Collins syndrome is characterized by malar and mandibular hypoplasia, down-slanting palpebral fissures, lower eyelid coloboma, auricular malformations with/without aural atresia, and conductive hearing loss. Although cleft palate and choanal atresia have been reported, cleft lip has not. Inheritance is autosomal dominant and is associated with mutations in the *TCOF1* gene on chromosome 5q32 in most individuals.

We present a 21-year-old Inupiat female with malar and mandibular hypoplasia, bilateral lower lid colobomas, preauricular ear tag, small cup-shaped ears, profound mixed hearing loss, choanal atresia, and normal intelligence. Her hands and feet appear normal. Three-dimensional facial CT images show bilateral infra-orbital defects, retrognathia, and hypertrophy of the zygomatic arch with an accessory bony projection of the lateral orbital walls. Immediate family history is notable for six healthy siblings, three miscarriages, and two infant deaths; a female with bladder exstrophy and a male infant with imperforate anus, multiple ear tags, orofacial cleft, and absent left eyelid. Review of extended family pedigree reveals additional male and female relatives with Treacher Collins-like syndrome. These include a first cousin-once-removed, a second cousin, and a newborn second cousin-once-removed; all with mandibulofacial dysostosis and bilateral cleft lip/palate anomalies. Two of the cousins presented in the newborn period with choanal atresia and one child also has a multicystic dysplastic kidney and imperforate anus. All four individuals are Inupiat Eskimo and live in remote neighboring fishing villages in Seward peninsula of Alaska.

We describe a novel form of mandibulofacial dysostosis with presumed autosomal recessive inheritance, normal intelligence, additional clinical features of primary palatal clefting and anal atresia, and a unique radiographic facial structure.

Cleidocranial dysplasia, parietal foramina, cutaneous lesions and anal atresia: A new genetic disorder. *R. Mendoza-Londono, D. Napierala, S. Carter, W. McCormack, Y. Chen, E. Munivez, K. Wakui, L. Shaffer, B. Lee.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Cleidocranial dysplasia (CCD, MIM # 119600) is an autosomal dominant disorder characterized by hypoplastic or absent clavicles and delayed closure of fontanel. CCD is caused by mutations in the Runt domain transcriptional factor RUNX2, which is required for both osteoblast differentiation and chondrocyte maturation. We present a patient referred for evaluation in the newborn period because of dysmorphic features and imperforate anus. Examination revealed mid-face hypoplasia, frontal bossing with large anterior and posterior fontanel, open metopic suture extending down to the nasal bridge, imperforate anus, hypoplastic clavicles, and rocker bottom feet. The skin showed papules over the brow and posterior hairline. Chromosome analysis revealed normal female karyotype, 46,XX. Skeletal survey showed poor ossification of the parietal bones, hypoplasia of the sphenoid bone and of the mid facial bones and absence of the medial aspects of the clavicles. Head CT confirmed wide metopic suture and bi-parietal foramina as well as bicoronal synostosis. Spinal, renal and bladder US, and MRI of the brain were normal. Echocardiogram showed PFO and mild right ventricular hypertrophy. The skin lesions progressed and at four months of age were found on the face and extremities as papules and ruptured vesicles with an erythematous indurated border, in a psoriasis-like distribution. They did not respond to treatment with topical steroids. Skin biopsy of one of the lesions showed lichenoid dermatitis with eosinophils. Mutation analysis of the RUNX2 gene and other candidate genes failed to identify a mutation in this patient. FISH studies excluded deletions of the same genes. To this date, two families with affected sibs with a similar spectrum of findings have been described in the literature. We contend that our patient has a similar phenotype and together they constitute a CCD-related condition that is inherited in autosomal recessive fashion. We hypothesize that this condition may be caused by mutations in a novel protein that regulates the well-known context dependent function of RUNX2.

Survival with Schimke immuno-osseous dysplasia. *S. Lou*¹, *P. Lamfers*², *N. McGuire*², *C. Boerkoel*¹. 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Mercy Pediatrics and Adolescent Clinic, Clear Lake, IA.

Schimke immuno-osseous dysplasia (SIOD, MIM 242900) is an autosomal recessive pleiotropic disorder caused by mutations in *SMARCAL1*. Classically SIOD has been divided into an infantile or severe early-onset form and a juvenile or milder later-onset form, but based on data we have collected from 33 families, we suggest the disease severity and onset follows continuum such that patients with early onset and severe symptoms usually die early in life whereas those with later onset and mild symptoms survive into adulthood if treated with renal dialysis or transplantation. Severity and age of onset of symptoms do not, however, invariably predict survival because we also report a patient who has survived to 20 years despite having a homozygous *SMARCAL1* nonsense mutation and relatively severe early-onset disease. These observations provide not only an overview of the longevity of SIOD patients but also show that some severely affected patients can survive to adulthood.

New Phenotypes and Diagnostic Criteria for Alström Syndrome Based on Evaluation of 134 Cases. J.D.

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Alström Syndrome is characterized by retinopathy and blindness, neural deafness, obesity, insulin resistance, type 2 diabetes, hypertriglyceridemia, normal intelligence, and no polydactyly. Multiple organ failure (including cardiac, hepatic, and renal) occurs as patients age. Diagnosis can be confounded by the delay in appearance, progressive nature and variable clinical expression of this rare syndrome. Awareness of the complete phenotype is necessary for an earlier, more accurate diagnosis. Here we describe additional features including pulmonary, urological, neurological, developmental, and gastrointestinal dysfunction in 134 cases (69 female; 65 male, 2w-42y). Pathological work-ups on five patients are presented. We obtained clinical data through review of all available medical records, physician interviews, and standardized questionnaires administered to parents. Cardiomyopathy in 63% of patients fell into two distinct groups: a) infant onset, and b) adolescent/adult onset. Minor to severe urological dysfunction occurred in 65 patients (55%). Persistent pulmonary symptoms (bronchitis, asthma, chronic obstructive pulmonary disease, acute respiratory distress syndrome) occurred in 68 patients (58%). Twenty-seven patients had gastrointestinal reflux (23%). Neurological symptoms in 39% included absence seizures, Asperger's-like behaviors, clonic tics, or motor/language delays. Pathological findings include fibrotic infiltration in multiple organ systems: kidney, liver, lung, and heart. Further study of the clinical diversity will contribute to a better understanding of this complex disorder and aid physicians in making an early and accurate diagnosis for Alström Syndrome patients.

Novel X-linked Iron-Storage Disorder with Apparent Neurodegeneration and Skin Involvement. *J.M. Opitz¹, J.C. Carey¹, M.J. Bamshad¹, K. Swoboda², H. Zhou³, E. Gilbert-Barness⁴.* 1) Dept Ped, Div Medical Genetics, Univ Utah, Salt Lake City, UT; 2) Dept Ped, Div Pediatric Neurology, Univ Utah, Salt Lake City, UT; 3) Dept Ped, Div Pathology, Univ Utah, Salt Lake City, UT; 4) University of South Florida Department of Pathology, Tampa General Hospital, Tampa, FL.

We report on a unique family segregating for a previously unreported X-linked condition involving CNS and skin, and affecting 2 younger brothers in a sibship of 7 and their nephew, born to their older sister. Carriers are apparently completely normal; however, the mother of the original sibship died of metastatic breast cancer within a day of her older affected son. Affected boys are born normally without intrauterine growth retardation and undergo apparently normal psychomotor development for 6 months; a premonitory sign is a dolls eye/head bobbling phenomenon shortly before the onset of seizures at 7 months with subsequent relentless downhill course, loss of all psychomotor maturation and vision, with only an occasional smile in response to touch and voice. MRI of CNS and results of all neuro-metabolic tests are normal; however, one of the brothers developed microcephaly. By the time of death, an initially ichthyosis-like skin rash on trunk and axillae had become a histologically psoriasis-like disorder. Studies of fatty alcohol NAD activity is normal, ruling out Sjgren-Larsson syndrome. The affected nephew has a large head; not (as yet) developed skin involvement. Death is due to pneumonia. On autopsy of the younger brother a massive degree of iron deposition was found in all viscera, most strikingly in liver; in his brain there was a scattered loss of neurons. No linkage to the sideroachrestic anemia/MR locus was found.

BRITTLE CORNEA SYNDROME AND ITS DELINEATION FROM THE KYPHOSCOLIOTIC TYPE OF EHLERS-DANLOS SYNDROME: REPORT ON 23 PATIENTS AND REVIEW OF THE LITERATURE. B.

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The Brittle Cornea Syndrome (BCS) is a rare, generalized connective tissue disorder characterized by ruptures of the cornea without adequate trauma, keratoconus or keratoglobus, blue sclerae, hyperelasticity of the skin without fragility, and hypermobility of the joints. It is inherited as an autosomal recessive trait but its genetic defect remains elusive. We present 23 (11 male) patients from 13 nuclear families followed at the King Khaled Eye Specialist Hospital, Riyadh, Saudi Arabia. They were aged 3 to 28 years at last follow-up. A total of 28 events of ruptures were noted in 15 patients (8 male); 9 among them had bilateral ruptures, 8 unilateral ruptures (4 of the right cornea), and 2 re-ruptures 2 and 4 years after surgery; 6 patients (aged 3-21 years) had no ruptures. We describe the natural history of our cases and discuss them with those reported in the literature. Because of similarities of the BCS with the kyphoscoliotic type of the Ehlers-Danlos syndrome (EDS VI) both disorders have been confounded. Here, we show biochemically that all patients with BCS had biochemical findings reflecting normal lysyl hydroxylase activity such as normal urinary total pyridinoline ratios (lysyl pyridinoline / hydroxylysyl pyridinoline; mean 0.23, range 0.16-0.38, n=18; controls: 0.20 ± 0.10 , range 0.10-0.38; EDS VI: 5.97 ± 0.99 , range 4.3-8.1, n=17) and/or normal electrophoretic migration of the α -chains of various collagens produced by the fibroblasts. We conclude that the BCS is an entity distinct from that of the kyphoscoliotic type of Ehlers-Danlos syndrome which is caused by a deficiency of the enzyme collagen lysyl hydroxylase and has a much poorer prognosis (Steinmann et al.: The Ehlers-Danlos syndrome. In: *Connective Tissue and Its Heritable Disorders* (P. M. Royce and B. Steinmann, eds), 2nd ed., pp. 431-523, Wiley-Liss, New York, 2002).

Interstitial del 20p (Alagille syndrome) associated with imperforate anus and hypopituitarism. *R.A. Pinter¹, W.A. Hogge¹, U. Surti^{1, 3}, E. McPherson^{1, 2}.* 1) Dept Genetics, Magee-Womens Hosp Pittsburgh, PA; 2) Dept Pediatrics, Childrens Hosp Pittsburgh, PA; 3) Dept Pathology, University of Pittsburgh, PA.

A 2950-gram male was born at 38 weeks to a 29 year-old G6P5 woman by induced vaginal delivery due to prenatal diagnosis of right multicystic dysplastic kidney, left hydronephrosis, and inability to identify sex on ultrasound. Upon newborn examination he was found to be a male with a small non-rugated, non-pigmented scrotum, micropenis, and testes in the inguinal canals, and with an imperforate anus. He had a broad forehead, but otherwise did not have the facial appearance of Alagille syndrome. His anterior fontanel was large and full. His nipples were wide set, and he had excess nuchal skin, clubfoot, long fingers with hyperconvex nails and hypoplastic toenails, and decreased central tone. Eye examination was normal. Hypoglycemia was noted at birth, and low cortisol and hypothyroidism with low TSH were subsequently found. He developed direct hyperbilirubinemia over the next few days. X-rays showed butterfly vertebrae and sacral malformation. Abdominal ultrasound confirmed the prenatal findings, and showed no hepatic anomaly. A brain MRI showed severe maldevelopment of the pituitary gland, and an echocardiogram was remarkable for two small ventricular septal defects. Blood karyotype was 46,XY,del(20)(p11.23-p13). His parents' karyotypes were normal.

The proband exhibits many features of Alagille syndrome, known to be associated with the deletion of *JAG1* at 20p12. This is the first case reported with imperforate anus and hypopituitarism in association with deletion in this area.

Novel CNS syndrome and ectodermal dysplasia. *R. Zannolli¹, C. Miracco², M.M. de Santi², F. Macucci¹, R. Di Bartolo¹, L. Serracca¹, F. Giannini³, A. Malandrini⁴, P. Galluzzi⁵, S. de Robertis⁶, S. Cavani⁹, T. Hadjistilianou⁶, R. Perotti⁷, T. Doldo⁸, M. Fimiani⁷, R. Giorgetti⁸, M. Pierluigi⁹.* 1) Dept. of Pediatrics, Obstetrics and Reproductive Medicine, Section of Pediatrics, Policlinico Le Scotte, University of Siena, Siena, Italy; 2) Dept. of Pathological Anatomy and Histology, Policlinico Le Scotte, University of Siena, Siena, Italy; 3) Dept. of Neuroscience, Section of Neurology, Policlinico Le Scotte, University of Siena, Siena, Italy; 4) Dept. of Neurological Sciences, Policlinico Le Scotte, University of Siena, Siena, Italy; 5) Neuroradiology Unit, Azienda Ospedaliera Senese, Policlinico Le Scotte, Siena, Italy; 6) Dept. of Ophthalmology and Neurosurgery, Policlinico Le Scotte, University of Siena, Siena, Italy; 7) Dept. of Dermatology, Policlinico Le Scotte, University of Siena, Siena, Italy; 8) Dept. of Orthodontics, School of Dentistry, University of Siena, Policlinico Le Scotte, Siena, Italy; 9) Human Genetics Laboratory, Galliera Hospital, Genoa, Italy.

We report a novel CNS syndrome in a 5-year-old male, with a tower-like skull, cerebral hemisphere asymmetry, retinal dystrophy, joint laxity, temporal triangular alopecia, and defective skin, hair and mammalian gland development. Interestingly, a skin biopsy showed a reduced number of hair follicles, hypoplasia of sebaceous glands, abnormal position of eccrine sweat glands, areas lacking filaggrin, hair follicle hyperkeratinization, signs of degeneration, aberrant melanosomes, and collagen fiber disorganization. Before the skin biopsy, diagnosis of this complex syndrome was delayed for several years. For some aspects, this syndrome was similar to Gomez-Lopez-Hernandez syndrome [MIM 601853] for including the same skull shape, a patch of alopecia, and clynodactyly; however it was quite different for the absence of ataxia, trigeminal anesthesia, cerebellar anomaly, corneal opacities. We believe it is important to report this case, as it shows that CNS syndromes are frequent associated with ED traits, often unrecognized. They sometimes present in very intriguing forms, not always matching classifications and previous descriptions.

Overlap of clinical features of Kabuki Syndrome and Branchio-Oto-Renal Syndrome: Contiguous gene syndrome, locus heterogeneity or coincidence? *M.S. Williams*. Dept Pediatrics, Gundersen Lutheran Medical Ctr, La Crosse, WI.

An 8-year-old boy was diagnosed with Kabuki syndrome on the basis of characteristic clinical features. Additionally, he was found to have bilateral preauricular pits, unilateral high frequency sensorineural hearing and unilateral renal agenesis. A review of Kabuki syndrome indicates that preauricular pits are seen in 39% of patients and clinically significant hearing loss is seen in 32% of patients. Some patients show a high frequency sensorineural hearing loss associated with Mondini dysplasia, while conductive and mixed losses are also seen. The correlation between the presence of preauricular pits and hearing loss has not been studied in Kabuki syndrome. Renal anomalies, including horseshoe kidney and renal dysplasia have been reported in Kabuki syndrome, but no cases of unilateral renal agenesis have been previously described. Branchial clefts or cysts also have not been reported. Several cases of Kabuki syndrome have been associated with chromosomal anomalies, but none of these involve chromosome 8. Autosomal dominant inheritance has been reported in several families. Branchio-oto-renal (BOR) syndrome is a well-characterized autosomal dominant syndrome consisting of the association of branchial arch anomalies, hearing loss (sensorineural, conductive or mixed) and renal anomalies. Mondini dysplasia and ossicular chain anomalies have been reported in BOR syndrome. Mutations in the EYA1 gene located at chromosome 8q13.3 are found in 30-70% of classic BOR patients. The phenotypic overlap of Kabuki and BOR syndromes is intriguing, and suggests that the EYA1 locus should be evaluated for microdeletions in the subset of Kabuki patients with preauricular pits and hearing loss, despite the fact that the few patients with such a deletion have not exhibited typical features of Kabuki syndrome. Exclusion of 8q13.3 in some families with autosomal dominant Branchio-otic syndrome has been observed, but none of these families has shown renal involvement. Correlations between preauricular pits, hearing loss, middle and inner ear structural malformations and renal abnormalities in Kabuki syndrome patients should be sought.

A dominantly inherited syndrome with frontonasal malformation : variant of Teebi hypertelorism syndrome or new entity ? A. Verloes^{1,2}, C. Baumann¹. 1) Clinical Genetics Unit, Hosp Robert Debre, Paris, France; 2) INSERM E9935.

We report a 3 generational family with 4 affected patients, showing dominant transmission of hypertelorism and other anomalies of the periorbital area, normal stature, brachydactyly, and variable anomalies : low birthweight (3/4), preauricular anomalies (tags 1/4 and pits 3/4), clefting (2/4), herniae (inguinal 2/4, diaphragmatic :1/4). Low normal mental development was present in 3 patients, whereas moderate mental retardation with autistic behaviour was present in the proband. Facial anomalies resemble craniofrontonasal dysplasia, but absence of craniosynostosis and presence of anomalies in other systems allow easy distinction. Teebi hypertelorism syndrome (hypertelorism, broad nose with broad basis and bridge, abnormal palpebral slit, wide set, arched eyebrows) appears the most suitable diagnosis for this family, although presence of several extra features could justify the delineation of a new entity within the frontonasal community of syndromes.

Syngnathia, vertebral segmentation defect and mental retardation : a new case of Dobrow syndrome. *M. Raoul¹, D. Geneviève¹, Y. Sznajer¹, C. Baumann¹, J. Lombet², V. Rigo², JP. Misson², A. Wilmont³, A. Verloes¹.* 1) Service de Génétique et INSERM E9935, Hopital Robert Debré, Paris, France; 2) Département de Pédiatrie, Hopital La Citadelle, Liège, Belgique; 3) Service de Chirurgie Maxillo - faciale, Hopital La Citadelle, Liège, Belgique.

Congenital bony fusion of the maxilla and mandible is a rare condition. Dawson et al (1997) proposed to subdivide syngnathia in type 1 (isolated syngnathia) and type 2 (syndromal syngnathia), among which type 2a is associated with aglossia and type 2b with agenesis or hypoplasia of the proximal mandible. Most of the reported cases (about 20 to date) are consistent with either Hanhart (aglossia - adactylia) or Goldenhar (facioauriculovertebral) syndromes.

We report a case of multiple defects associated with bilateral syngnathia in a female newborn. Bony fusion of the left mandible and maxilla was associated with severe dysplasia of the left temporomandibular joint and hypoplasia of the mandible and maxilla. This was confirmed by 3D reconstruction of facial CT scan. She had microcephaly, up-slanted palpebral fissures, unilateral coloboma of the iris, small dysplastic ears, cleft palate, severe retrognathism, and skeletal anomalies, namely hemivertebrae and costal fusions. Neurological examination revealed persistent hypotonia, nystagmus and neurosensitive impairment with normal cerebral CT scan. Follow up at 18 months showed developmental delay and seizures. Blood chromosomes were normal and there was no toxic exposure during pregnancy.

This patient is almost similar to the patient described by Dobrow (Syndrome identification case report 96, *J Clin Dismorphol.* 1983;1(2):5-7). This allows to delineate a new MCA/MR syndrome with syngnathia distinct from the types defined by Dawson.

Methylation pattern of human GRB10 (growth factor receptor binding protein) gene in patients with Silver-Russell syndrome or intrauterine growth retardation. *H-W. Yoo^{1,2}, S-S. Kim², G-H. Kim²*. 1) Dept Pediatrics, Asan Medical Ctr, University of Ulsan College of Medicine, Seoul, Korea; 2) Medical Genetics Clinic & Laboratory, Asan Medical Center, Seoul, Korea.

Silver-Russell syndrome(SRS) is characterized by intrauterine growth retardation, postnatal growth failure, and typical dysmorphic features including a small triangular facies, fifth-finger clinodactyly, and asymmetry of head and limbs with a relatively large, prominent forehead. The GRB10 gene, mapped on chromosome 7p11.2-p12, has been suggested as one of the strong candidate genes for SRS. GRB10 gene, which is known to be imprinted in both mouse and human in a paradoxical way, codes for growth factor receptor binding proteins. Overexpression of GRB10 leads to growth suppression by inhibiting tyrosine kinase activity. In this study, efforts was focused on the clarification of methylation pattern in genomic DNA of patients with SRS or intrauterine growth retardation(IUGR) without postnatal catch-up growth. Genomic DNA was isolated from peripheral lymphocytes of 15 patients with SRS and 23 patients with IUGR. After modification of genomic DNA with bisulfite treatment, methylation specific nested PCR was carried out using two sets of primers, one set specific for the bisulfite modified 5' UTR region of GRB10, the other for nested PCR with modified primers to create MboI restriction site for an imprinted allele derived from paternal origin. After nested PCR of bisulfite modified genomic DNA, the PCR product was subsequently digested with MboI restriction enzyme so that allelic pattern could be differentiated. Among 38 patients(15 SRS and 23 IUGR) screened, only 3 patients(1 SRS and 2 IUGR) showed mono-allelic methylation pattern. One out of 15 patients with SRS revealed a paternal mono-allelic pattern. Two out of 23 patients with IUGR showed a paternal and maternal mono-allelic pattern respectively. In these patients, DNA sequencing at methylation specific region using bisulfite-modified PCR product and STR genotyping using D7S481, D7S691, D7S2428, D7S2445, and D7S636 were undertaken. In conclusion, loss of maternal or paternal allele of GRB10 is associated with SRS and IUGR.

A new autosomal dominant skeletal dysplasia caused by a mutation of the *COMP* gene. *H.M. Saal¹, J.A. Johnson¹, J.W. Longshore², R.S. Lachman³.* 1) Div Human Genetics, Cincinnati Hosp Med Center, Cincinnati, OH; 2) DNA Diagnostic Laboratory, Carolinas Medical Center, Charlotte, NC; 3) Medical Genetics-Birth Defects Center, Cedars-Sinai Medical Center, Los Angeles, CA.

Mutations of the gene encoding the cartilage oligomeric matrix protein (COMP) have been associated with pseudoachondroplasia and multiple epiphyseal dysplasia (MED). We have seen 3 members of a multiplex family with disproportionate short stature in 13 individuals. X ray survey performed in childhood on one adult member showed multiple epiphyseal dysplasia. The proband, a 13 month old boy presented with short stature. He was 48.3 cm at birth. At 4 months his length was less than 5th centile (58 cm) and at 13 months his length was 67.5 cm (50th centile for 6 months). The patient's mother and aunt both had disproportionate short stature with micromelia and brachydactyly. Heights of affected males ranged from 153-157 cm, and affected females 144-147 cm. There was no history of significant joint pain or limitation of ambulation, except for mild osteoarthritis. No individual required any orthopedic interventions. X rays of the proband showed multiple changes, including brachydactyly, abnormal carpals and metacarpals with premature fusion of the epiphyses of the phalanges and metacarpals. The vertebrae were flattened with posterior wedging and interpedicular narrowing in the lumbar region. Analysis for a *COMP* gene mutation by direct DNA sequencing demonstrated a missense mutation within exon 11 at position 1181 with an A to C substitution predicting an amino acid change of N386H (asparagine to histidine). Pseudoachondroplasia is an autosomal dominant condition that usually presents in the second year of life with deceleration of linear growth and progressive degenerative changes in the hips and knees. Severe osteoarthritis is common, as is scoliosis. Adult height ranges from 82-130 cm. The MEDs are more variable. The two most commonly described are the Fairbank type and the milder Ribbing type. However in the MEDs, body proportions tend to be normal except for the presence of brachydactyly. Our family clearly does not fit well within either phenotypic group, sharing features of both.

True thymic hyperplasia in an infant with Beckwith-Wiedemann Syndrome. *I. Scala¹, R. Della Casa¹, V. Fiorito¹, A. Riccio², G. Sebastio¹, G. Andria¹.* 1) Department of Pediatrics, Federico II University, Naples, Naples, Italy; 2) Second University of Naples, Naples, Italy.

Beckwith-Wiedemann syndrome (BWS) is a variable disorder whose major features include omphalocele, macroglossia, and macrosomia. Overgrowth may be generalized or segmental and may involve selected organs such as kidneys, pancreas, adrenal glands, liver. True thymic hyperplasia is a rare childhood disorder consisting of increased organ volume by excessive growth of normal thymic structures. Thymic dysplasia has been reported in association with BWS, but, in these cases, the thymus was hypoplastic and immunodeficiency was associated. Up to now, the association of true thymic hyperplasia with BWS has been reported only in one case. A new case of true thymic hyperplasia in an infant with BWS is here reported. Case report. A five months old girl came to our attention for crossed segmental hemi-hyperplasia, overgrowth, umbilical hernia, tongue hemi-hypertrophy, enlarged kidneys. The clinical diagnosis of BWS was confirmed by molecular analysis of the BWS chromosomal region 11p15.5, which showed hypomethylation of the KvDMR1 locus and hypermethylation of the H19 locus. An occasional chest x-ray revealed a mediastinic mass occupying the superior-posterior mediastinum. An ultrasonic examination of the mediastinum did not support thymic neoplasia or cysts. MRI showed an expansive homogeneous mass with a signal identical to a normal thymic tissue. The mass had caused hypoplasia of the pulmonal apex, dislocated the superior vena cava and reached the right paravertebral region. The child did not present symptoms related to the thymic mass. Conclusions. Here we describe a new case of BWS associated with true thymic hyperplasia. Although macroglossia, omphalocele and gigantism are the most frequent manifestations of BWS, the syndrome is often variable. Patients were described who lacked any of the three major manifestations but showed visceral histological lesions. The finding of thymic hyperplasia in this BWS case suggests that it might not be a rare finding in this syndrome. Hence, true thymic hyperplasia might be looked for in those infants with uncertain clinical diagnosis of BWS.

A Study of the Neuropsychological Manifestations in Children with 22q11 Deletion Syndrome. *V. Shashi¹, M.N. Berry¹, T.R. Kwapil².* 1) Pediatrics, Wake Forest Univ Sch Med, Winston-Salem, NC; 2) Psychology, University of North Carolina at Greensboro, NC.

Schizophrenia is increasingly viewed as a neurodevelopmental disorder, but prospective studies of the etiology and premorbid manifestations are hampered by the lack of a homogeneous study population. Chromosome 22q11 deletion syndrome (22q11DS) is an autosomal dominant microdeletion syndrome, associated with congenital abnormalities, cognitive impairment and a markedly increased incidence (40%) of schizophrenia and other major psychoses in late adolescence/adulthood. Due to the relatively recent discovery of the relationship between 22q11DS and psychoses, there is a paucity of knowledge regarding the pathogenesis, neurobiology and the clinical course of psychoses in these individuals. We carried out a pilot study to gather cross-sectional data on 10 children with 22q11DS and 10 age, gender and socioeconomically matched healthy control subjects. This cross-sectional study assessed neurodevelopmental history (including pre- and perinatal complications), pedigree data, medical status, intellectual ability, psychometric/biobehavioral/neurocognitive measures of risk for schizophrenia and structural brain abnormalities by morphometric analyses. Our preliminary data indicate that children with 22q11DS exhibit higher rates of anxiety disorders and AD/HD, in addition to cognitive deficits. On preliminary qualitative brain MRI analysis, structural abnormalities are common, such as a small cerebellar vermis, white matter hyperdensities and midline deviations such as cavum septum pellucidum/vergae (4/7 patients). Our findings are suggestive of increased rates of neuropsychological and neuroanatomical abnormalities in children and adolescents with 22q11DS. Further characterization of these abnormalities in the proposed cross-sectional study will form the basis of a future longitudinal study of risk for schizophrenia and other psychoses in these children. The future longitudinal study would increase the understanding of the causative mechanisms, specify the clinical phenotypes and identify biopsychosocial factors that may increase or decrease the likelihood of developing psychosis.

Postaxial acrofacial dysostosis in two brothers: Miller syndrome or a new entity? *G. Viot¹, S. Fert², A. Munnich¹, V. Cormier¹*. 1) Department of Genetics, Necker-Enfants malades , Paris, France; 2) Service de cytogenetique, Centre Hospitalier de Chambéry, France.

The acrofacial dysostosis syndromes are a heterogeneous group of disorders combining defects of craniofacial and limb development. Usually, the difference between these syndromes lies in the preaxial (namely Nager syndrome) or postaxial (namely Miller syndrome) site of the defect. But the delineation of these different entities is sometimes difficult because of a great clinical variability. Therefore, we report the cases of two brothers with ulnar ray deficiency, camptodactyly, shortened forearms, absence of toes from the lateral border of the feet and bifid first toes. Facial dysmorphism consisted of mild malar hypoplasia responsible for antimongoloid slant of the palpebral fissures, cup-shaped ears, lower lid ectropion, broad nose with unilateral choanal atresia, cleft palate and square chin . They also presented hypoplastic pectoral girdle, cryptorchidism, hearing problems and feeding difficulties. The first boy died shortly after birth because of pneumopathy. The second is 18 years-old and has normal intelligence. Limb defects presented by these two brothers are consistent with the Miller syndrome but the facial dysmorphism and the absence of other skeletal abnormalities suggest that this familial form of postaxial acrofacial dysostosis could be a new entity with an autosomal recessive or an X-linked inheritance.

Defining the clinical spectrum of Alveolar Capillary Dysplasia (ACD). *P. Sen*¹, *C. Langston*^{1,2}, *B.A. Bejjani*³. 1) Pediatrics, Baylor College of Medicine, Houston, TX; 2) Pathology, Baylor College of Medicine, Houston, TX; 3) Mol/Hum Genetics, Baylor College of Medicine, Houston, TX.

Alveolar Capillary Dysplasia (ACD) is a rare and lethal developmental anomaly of the pulmonary vasculature. It is described as the failure of formation of the normal air-blood diffusion barrier in the newborn lung. ACD is usually associated with misalignment or displacement of the pulmonary veins. The disease presents very early in infancy, usually after a few hours of normal breathing. Infants become critically ill very rapidly in the first days of life with severe hypoxemia and pulmonary hypertension. The disease is uniformly lethal. Standard therapies include mechanical ventilation, high concentrations of inspired oxygen, inhaled nitric oxide and ECMO support. These therapies may prolong life by days to weeks, but have led to no long-term survival. We have established a close working relationship with the ACD Association, the only parent support organization and have already collected 29 families, each with one or more infant with ACD in preparation for positional mapping and eventually cloning the ACD gene(s). Clinical records and pathological samples from affected individuals, and DNA from affected and unaffected individuals were obtained. Our review of the pre and postmortem records of affected individuals allows us to define better both the natural history of this condition and the associated anomalies with the ACD phenotype. Our collection of families corroborates the possible recessive nature of this condition and provides additional data for genetic and prenatal counseling. Analysis of the various associated anomalies allows us to formulate a hypothesis regarding possible candidate genes and/or signaling pathways. The material collected here represents the largest cohort of ACD patients ever assembled. It also allows for positional mapping of the putative ACD gene as a first step towards understanding this condition. Understanding the clinical spectrum of ACD and cloning an ACD gene have implications for counseling, for prenatal testing, and for understanding the molecular pathophysiology of ACD and other organ malformation that are associated with this condition.

Chromosome 22q11 deletion syndrome and sclerocornea in an infant boy. *M.L. Raff¹, A.L. Mitchell², W. Robinson³, K.A. Leppig⁴, A.H. Weiss⁵.* 1) Div. of Genetics and Development, Children's Hospital and Regional Medical Center and the Dept. of Pediatrics, University of Washington, Seattle, WA; 2) Div. of Medical Genetics, Dept. of Medicine, University of Washington, Seattle, WA; 3) Dept. of Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 4) Group Health Cooperative, Seattle, WA; 5) Dept. of Ophthalmology, University of Washington, Seattle, WA.

Anterior segment dysgenesis of the eye has not previously been reported in an individual with a 22q11.2 deletion. We report a male child with congenital sclerocornea with mild microphthalmia and a FISH-positive 22q11.2. A moderately-sized ventricular septal defect was documented at 3 months of age. At 5 months, he was admitted to hospital for growth failure, at which time his length was at the 50th centile for a 2 month old, his weight was at the 50th centile for a 1-1/2 month old, and his OFC was at the 5th centile. Karyotype performed at that time was normal, but FISH analysis was positive for the 22q11 deletion. The child later manifested global developmental delays, severe gastroesophageal reflux necessitating a Nissen fundoplication, and small bowel obstruction requiring. Corneal opacification precluded visualizing the posterior segment of the eye. However, B-scan ultrasound revealed a clear vitreous and attached retina. ERG showed a normal photopic and scotopic response. Head CT scan at 5 month of age confirmed mild microphthalmia with a globe axial length of 16mm bilaterally. Visual acuity evaluations found no consistent response to the low vision Teller cards, implying a visual acuity of less than 20/2000. Molecular analysis demonstrated heterozygosity of flanking microsatellite markers D22S427 and D22S425, suggesting that this child's deletion could not be significantly larger than the usual deletion associated with 22q11 deletion syndrome. While this represents a relatively severe case of the syndrome, all of the features described here other than the sclerocornea are previously described associations with a 22q11.2 deletion. Thus, we propose that anterior segment dysgenesis be added to the list of associated findings in 22q11 deletion syndrome.

Subtle 2p24-p25 deletion in a boy with Pierre Robin sequence and developmental delay. *P. Gosset¹, C. Ozilou¹, G. Baujat², V. Abadie², M. Picq¹, M. Vekemans¹, S. Lyonnet¹, MC. de Blois¹.* 1) Dept Genetics, Hopital Necker-Enfants Malades, Paris, France; 2) Dept Pediatrics, Hopital Necker-Enfants Malades, Paris, France.

Cryptic rearrangements of subtelomeric chromosome regions are found in about 7% of children with unexplained mental retardation. Here we report the presence of a subtelomeric deletion of the short arm of chromosome 2 in a patient with severe Pierre Robin sequence and developmental delay.

The proband is the first child of young and healthy parents. During pregnancy, amniocentesis was performed because of the discovery of IUGR and Pierre Robin sequence. The karyotype was interpreted as normal. Pregnancy was continued. At birth weight was 1670g and length 40cm. Severe Pierre Robin sequence was noted. Proband was seen at 18 months of age because of developmental delay and dysmorphic features including large forehead, hypertelorism, narrow palpebral fissures, epicanthal folds, small nose and microstomia. Microretrognathism was severe and the ears were low set. The new High resolution R and G banding, was normal. A subtelomeric screen using Multiprobe-T System (Cytocell, UK) was performed. It revealed a deletion of the distal end of a chromosome 2p. This deletion was confirmed using appropriate FISH probes.

The present study illustrates the importance of subtelomeric screen in cases of unexplained mental retardation and dysmorphic features.

Two Filipino Cases With Rare Autosomal Trisomies. *B.C.V. Cavan*¹, *A. Milagrosa*², *E.M.C. Cutiongco*^{1,3}, *C.D. Padilla*^{1,3}. 1) Department of Pediatrics, University of the Philippines College of Medicine-Philippine General Hospital, Manila, Philippines; 2) Department of Pediatrics, Ilocos Training and Medical Regional Center, San Fernando City, La Union, Philippines; 3) Institute of Human Genetics, National Institutes of Health, University of the Philippines, Manila, Philippines.

Trisomies for autosomes other than chromosomes 21, 18 and 13 are noted to have severe consequences and inevitably result to fetal death in utero. There are rare instances wherein these other autosomal trisomies survive the neonatal period. We report two such cases. The first is a case of a 1-month old male born premature to a non-consanguineous Filipino couple. This case presented with intrauterine growth retardation, microcephaly, hypertelorism, epicanthic folds, midface hypoplasia, low set ears, multiple ventricular septal defects and a hypoplastic 5th distal phalanx. The diagnosis of trisomy 22 was confirmed in all cells examined by chromosomal analysis (G-banding). The second case is a 3-month old female born term to non-consanguineous Filipino parents. She presented with a high arched palate, low set ears, bifrontal hair whorls, unequal palpebral fissures, hypertelorism, limited movement of the left eye, overlapping fingers on the right hand (5th digit over the 4th digit, 2nd digit over the 3rd digit), large flat nipples with a hypopigmented central area of the areola, a sacral dimple, and a wide gap between the first and second toes. Chest radiographs showed eleven pairs of ribs. Chromosomal analysis by G-banding confirmed the diagnosis of Trisomy 11. To our knowledge, there are no other case reports of trisomy 11 in literature. Mosaicism is not discounted as a possible cause for the prolonged survival of our patient. These cases add to the world literature on autosomal trisomies in liveborns.

CPO1: A Cleft Palate Gene on 2q32.3. *D.R. FitzPatrick¹, J.A. Fantes¹, L. McLaren², N. McGill¹, H. Firth⁴, K. Williamson¹, J.P. Leek³, I.M. Carr³, S. Hassan³, P.J. Wightman¹, A.F. Markham³, D.T. Bonthron³.* 1) Cell & Molecular Genetics, MRC Human Genetics Unit, Edinburgh, UK; 2) Molecular Medicine Centre, University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU, UK; 3) Molecular Medicine Unit, University of Leeds, Clinical Sciences Building, St James's University Hospital, Leeds LS9 7TF, UK; 4) Department of Clinical Genetics, Addenbrooke's Hospital, Cambridge CB2 2QQ, UK.

We previously reported two unrelated children with very similar clinical phenotype and de novo apparently balanced chromosomal translocations involving 2q32.2 associated with isolated cleft palate - *Am J Hum Genet* 1999, 65:387-96. High resolution FISH mapping of these breakpoints demonstrated that one interrupts the transcription unit of the gene, KIAA1034 which we have renamed CPO1. The second breakpoint is 70 kb 3' of the polyadenylation signal in a region of non-coding DNA. This gene has telomeric-centromeric orientation and resides in a gene poor region of 2q32.3. The nearest confirmed neighbouring gene is 1.26 MB centromeric to the CPO1 polyadenylation signal. CPO1 has 12 exons which span 191kB of genomic DNA. It encodes a protein of 733 amino acids that has two CUT domains and a homeodomain and shows an extraordinary degree of evolutionary conservation with only 3/733 amino acid residues differing between the mouse and human peptides. Significant homology was also found to the chromatin binding protein SATB1 and the drosophila protein DVE1. Whole mount in situ hybridisation to mouse embryos using antisense riboprobes derived from two different regions of mouse CPO1 showed site- and stage-specific expression only in the developing palate. Haploinsufficiency for this gene appears to be responsible for the phenotype in at least one of these children and it is likely that the disruption of long-range gene regulation is involved in the second breakpoint. However, mutation analysis of 150 unrelated patients with isolated CPO did not reveal any potentially pathogenic changes or polymorphisms in the coding region suggesting that this gene is not a major contributor to the etiology of isolated cleft palate.

Identification of deletion of 4q34.2-qter in three generations of a family. *J.M. Cowan^{1, 2}, P.G. Wheeler²*. 1) Cytogenetics Laboratory, Tufts-New England Medical Ctr, Boston, MA; 2) Division of Genetics, Department of Pediatrics, Tufts University School of Medicine, Boston, MA.

The majority of microscopically visible deletions are characterized by dysmorphic features and often congenital abnormalities. Deletions of terminal 4q have been associated previously with facioscapulohumeral muscular dystrophy (FHD).

We have identified a family in which a deletion of 4q34.2-qter was present in three generations. The family was ascertained through identification of a terminal deletion of 4q in an amniotic fluid sample drawn for advanced maternal age. A level 2 ultrasound scan of the fetus did not reveal any abnormalities. A study of parental karyotypes revealed the same deletion in the mother of the fetus, while the father's karyotype was normal. The deletion was confirmed by telomere FISH. An expanded study of the mother's family identified the deletion in the proband's mother and brother. The phenotype of the affected individuals is characterized by difficulty in school, and though all are described as "slow" they are functioning adults, able to hold jobs.

Deletion of the distal part of 4q has not resulted in dysmorphic features in this family, though the affected individuals are intellectually slower than unaffected members of the family. In addition, the affected members have a history of miscarriage - the proband's mother had 5, and the proband's brother has had 2 losses. It is unclear at present whether this is directly related to the loss of distal 4q.

Four new patients with 2q terminal deletion syndrome: hemidiaphragmatic hernia as a novel finding and patterns of malformation among 47 reported cases. *K.A. Casas¹, H.J. Lin², C.N. Mikail¹, R.E. Falk¹.* 1) Medical Genetics/Birth Defects Ctr, Cedars-Sinai Medical Ctr, Los Angeles, CA; 2) Division of Medical Genetics, Harbor-UCLA Medical Ctr, Torrance, CA.

At least 43 individuals carrying an isolated, terminal deletion of chromosome 2q, with the breakpoint at or distal to region 3 band 5, have been described since 1983. We report four new subjects and examine phenotypes associated with breakpoints. Subject 1 (6 day-old male; breakpoint at 2q37.1) had multiple anomalies, including hemidiaphragmatic hernia, atrial septal defect, horseshoe kidney, and microtia. Subjects 2 and 3 (1 year-old male and 2 year-old male, respectively; breakpoint at 2q37.1) had global developmental delay, hypotonia, and unusual facies. Subject 4 (6 year-old female; breakpoint at 2q37.3) had developmental delay, autistic behavior, and unusual dentition. Whereas subtelomeric deletion polymorphisms within 2q are estimated to occur in five percent of the population, the frequency of larger, significant deletions is unknown. Such 2q deletions may be underdiagnosed. Among known subjects, developmental delay occurred in 95 percent of those surviving the newborn period. Other common manifestations were: hypotonia (51 percent); major malformation (32 percent); and autistic behavior (26 percent). Positions of breakpoints may be roughly associated with certain abnormalities. For example, a subject with a subtelomeric breakpoint displayed only autistic behavior and developmental delay. Facial features (e.g., frontal bossing, dysmorphic ears, short nose, flat nasal bridge) and short hands and feet were frequent in subjects with breakpoints at or proximal to 2q37.3. Reports of structural brain anomalies, horseshoe kidney, and Wilms tumor were limited to cases with the breakpoint within or proximal to 2q37.1. Cleft palate was reported only in subjects with proximal breakpoints (2q36 or 2q35). Hiatal hernia occurred in a subject who had a breakpoint at 2q37 (no sub-band identified), but no other diaphragmatic defect had been observed prior to Subject 1 above. Such information on breakpoints and phenotypes may be useful in counseling, if confirmed by further reporting.

Detection by PCR and FISH analyses of Y-chromosome specific sequences in genomic- and gonadal-DNA in patients with Ullrich-Turner syndrome. *F.J. Alvarez Nava, M.L. SOTO QUINTANA, L. BORJAS, A. ROJAS ATENCIO, M.A. SANCHEZ, E. FERNANDEZ, A. MORALES MACHIN.* Unidad de Genetica Medica, Univ del Zulia, Maracaibo Zulia, ZULIA, Venezuela.

Ullrich-Turner syndrome (UTS) is associated in 40-60% with 45,X karyotype, whereas the remaining cytogenetics findings have numerical or structural anomalies in second sexual chromosome (X or Y) in form pure or with mosaicism. It has been proposed that all females with UTS and 45,X karyotypes carry a cell line containing two sex chromosome at low level of mosaicism. This mosaicism is undetectable by standard cytogenetics analysis. Theoretically, this hidden mosaicism may has a Y chromosome. Gonadal dysgenesis seen in UTS patients is associated with gonadoblastoma when Y-chromosome is present in their genome. So it is crucial to carry out molecular analysis in these subjects to rule out Y-chromosome sequences. The present study was performed to examine the presence of Y-chromosome material in lymphocytes- and gonadal-DNA in UTS patients. Fifty-two Venezuelan patients were screened by polymerase chain reaction (PCR) to detect Y-chromosome fragments using ten Y-chromosome specific primer pairs. These primers are spanned all seven intervals of the Y-chromosome. Patients with virilization and/or with positive Y-chromosome sequences by PCR underwent gonadectomy. Fluorescence in situ hybridization (FISH) was used to confirm that chromosomes were Y-derived. For patients (two with 45,X/46,XY, one with 45,X/46,X,+mar, and one with 45,X karyotypes) were positive in lymphocytes-DNA to Y-sequences specific. All patients had Y chromosome material on gonadal-DNA. FISH confirmed their Y-chromosome origin. Only one of them (45,X) presented bilateral gonadoblastoma and she did not exhibit virilization. Our results suggest that the molecular analysis must be applied for detection of Y-chromosome in all UTS patients and do not to limit to UTS patients with cytogenetically identifiable Y-chromosome and/or virilization.

Sex chromosome aneuploidy diagnosed in younger children. *J. Bodurtha, U.T. Sundaram, C. Jackson-Cook.* VCU, Richmond, VA.

Early diagnosis of genetic conditions can help families plan for the future and address the worry associated with not having a diagnosis. Sex chromosome conditions in males are common, and include the classic Klinefelter syndrome, (46,XXY) as well as the rarer conditions with multiple X-chromosomes. Klinefelter syndrome is considered in the differential diagnosis in an adolescent with gynecomastia, and inadequate testosterone production, or in adult men being evaluated for infertility. Although developmental delay, such as mild delay in motor and language milestones, and learning difficulty are reported in sex chromosome aneuploidy conditions in males, they may not be strongly considered in children presenting with these symptoms leading to the under diagnosis in children. We report two male patients, who were diagnosed at 21 months and 15 months with sex chromosome aneuploidy. The first patient had developmental delay (especially speech), short stature, radio-ulnar synostosis, round face, and micropenis. He had been evaluated for metabolic disorders at two other institutions, and had received testosterone for micropenis with good response. We evaluated him after this intervention and ordered chromosome studies that revealed a 49, XXXXY complement. Some authors consider this condition to be a Klinefelter variant while others believe it is a distinct entity. The literature indicates a positive correlation between the number of X-chromosomes, and the severity of developmental delay and /or mental retardation. The second patient is an adopted child who we evaluated due to developmental delay (especially speech). He had a round face, hypertelorism, and shawl scrotum. Chromosome analysis revealed a 47, XXY complement. Sex chromosome aneuploidy, especially classic Klinefelter syndrome, may be frequently diagnosed prenatally but less so in infancy. These cases underscore the need to perform chromosome testing in male children who have delayed development, in particular speech impairment, and minimal distinctive findings, including small testes, micropenis and round face in infancy. An early diagnosis can benefit the patient through referral for early infant intervention as well as doing long term health and educational planning.

A study of congenital malformation associated with consanguinity in Khorasan Province, North East of Iran. *R. Akbarzadah¹, K. Ghodsi¹, G. Mamoori², A. Mohammadzadah², A. Shahfarhat², M. Akbarzadah¹, M. Gorgi Nejad¹, N. Khadem³, L. Hafizi³, S. Kadkhodaian³, N. Saghafi³, N. Lotfi², R. Akbarzadah¹, J. Parizadah².* 1) medical genetics, Ghaem hospital, Mashhad, Khorasan, Iran; 2) Pediatric, Ghaem hospital, Mashhad, Khorasan, Iran; 3) Gynecology, Ghaem hospital, Mashhad, Khorasan, Iran.

A study of congenital malformation associated with consanguinity in Khorasan province, North East of Iran. Reza Akbarzadeh¹, Kazem Ghodsi¹, Gholam Ali Mamoori², Ashraf Mohammadzadeh², Ahmad Shahfarhat², Marjaneh Akbarzadah¹, Mohsen Gorgi Nejad¹, Naierah Khadem³, Laili Hafizi³, Sima Kadkhodaian³, Nafisah Saghafi³, Nosrat Lotfi², Rehanah Akbarzadah¹, and Javad Parizadeh². Department of Medical Genetics¹, Department of Pediatric² and Department of Gynecology³, Ghaem and Emam Reza Hospitals, Faculty of Medicine, Mashhad University of Medical Sciences Mashhad Iran. First cousin marriage is common in Khorasan which is the biggest province in Iran. It is consisted of various people and cultures. We randomly studied 3624 marriage in 25 cities of this province, to investigate the role of first cousin marriage and genetic disorders. In this group consanguinity was present in 25.9%. Analyzing by gender demonstrated that 44.2% of patients were girls and 57.8% boys. Pedigrees were analyzed and showed that 33.2% of congenital abnormalities were born to consanguinity families. It also showed that death under one year old 10.3%, recurrent abortion 32.7% and other anomalies 57% including disorders of bone and connective tissues 30.3%, gastrointestinal tract 20.7%, central nervous system disorders 18.5%, kidney diseases 12.2%, congenital heart disease 6.7%, deafness 3.3%, blindness 2.5%, and skin disorders 3.8% were presented in patients. Etiological study showed that multifactorial inheritance was 42%, autosomal dominant 21.3%, autosomal recessive 4.3%, X-linked 2.6% unknown causes 15.8% and chromosomal abnormalities containing trisomy 21, 13 and 21/21 translocation 14%.

Gender ambiguity and fragile X: a new syndrome. *K.J. Griffin¹, T. Bei¹, J. Meck², L.-J. Wong², C. Bondy¹, C.A. Stratakis¹.* 1) UGEN/DEB, NICHD, NIH, Bethesda, MD; 2) Institute for Molecular & Human Genetics, Georgetown University, Washington DC.

We recently encountered a family with a novel condition: the concurrent presence of ambiguous genitalia and FXS in a 16-year-old male with severe mental retardation (MR) and a 29-year-old female with mild MR. Both were children of a 55-year-old woman with no MR but mild stigmata of FXS and paucity of axillary and pubic hair. The female child was born with ambiguous genitalia; cryptorchid testes were removed at birth. A third sibling, a 19-year-old female, was phenotypically normal and had normal hair. The mother and the 3rd sibling had a 46,XX chromosomal constitution; the father and the two affected siblings had 46,XY. Molecular analysis revealed normal androgen receptor (AR) by FISH and identical X in all 3 siblings by microsatellite markers; FMR-1 analysis showed that the mother was a carrier for a premutation; the affected children had a heterogeneous expansion up to 270 repeats in the male and 600 repeats in the female. The 46, XY female also had the premutation; she was an apparent mosaic, as was her mother. These findings were confirmed by EcoRI/EagI digests. AR sequencing of the 46,XY female showed no known disease-causing mutations; cDNA of the receptor (from peripheral lymphocytes) was also normal. However, the AR exon 1 glycine repeat track length was only 10 repeats, which has been associated with decreased AR function under certain conditions. This change was also present in the unaffected 3rd sibling, a normal 46,XX female. The present report identifies a family with sexual ambiguity and FXS. Apparent mosaicism for a mutation and premutation status of FMR-1 may explain the phenotypic differences between the 2 affected siblings. Mosaicism for FMR-1 gene mutation and/or other genetic defects may affect the expression or function of the AR gene carrying certain polymorphisms, or alter FXS phenotype to include sexual ambiguity.

ECTRODACTYLY IN THE GENETICS CLINIC. *B.G. Kousseff.* Pediatrics & Genetics, USF Regional Genetics Prog, Tampa, FL.

Ectrodactyly is encountered in mendelian traits as well as in rare chromosomal anomalies and as sporadic. The anomaly occurs during organogenesis and recently its pathogenesis is better understood (Wolpert, *Pediatr Res* 46:247-254, 1999). Retrospective analysis of the 51 probands with confirmed or suspected ectrodactyly evaluated at the USF genetics/dysmorphology and prenatal clinics between 1/2/82 and 12/31/00 is presented. The probands were part of the 38,706 probands/families evaluated during the period. They were retrieved using patient database. Ectrodactyly/split hand was one of the 3 primary diagnoses. Four of the 51 were no shows in the clinic and were excluded. There were 29 males and 18 females. Twenty-eight of 47 were Caucasian, 10 Hispanic, and 9 African-American mismatching the racial ratio of the population of West Central Florida. Typical lobster claw anomaly type I was present in 19 and atypical in 28. Two had chromosome anomaly, t(13q14q) paternal with maternal class A 1 diabetes mellitus in one and another with complex chromosome rearrangement with 6 breaks on chromosomes 2,3,5,11, and 13 resulting in interstitial deletion 13q. (Kousseff et al. *Clin Genet* 42:135-142 1992). Three probands had mothers with diabetes mellitus but no other anomalies to diagnose diabetic embryopathy. Twelve of the 47 had positive family history indicative of autosomal dominant trait with reduced penetrance. Eighteen of the 47 had associated anomalies implying syndromes. Among them were 3 probands with ectrodactyly-ectodermal dysplasia-cleft palate. This study showed that ectrodactyly has 1. varied phenotypes difficult to classify and 2. is genetically heterogeneous. As to a candidate gene only the probands with chromosomal abnormalities implied chromosome 13.

Severe psychomotor retardation associated with mosaic Down syndrome and mosaic Turner syndrome involving trisomy 21, monosomy X and ring X cell lines. *M.T. Jodah¹, L.L. Estabrooks², K. Clark², B.G. Kousseff¹*. 1) Dept. of Pediatrics, USF Regional Genetics Program, Tampa, FL; 2) Genzyme Genetics, Tampa, FL.

CASE REPORT: A three-year old, Mexican female (IV) was referred to the genetic clinic with a diagnosis of Down syndrome, diagnosed prenatally via amniocentesis for advanced maternal age; the cytogenetic report was unavailable. The phenotype of IV consisted of intermittent nystagmus, synophrys, patent ductus arteriosus, minimal upslant to the palpebral fissures, epicanthal folds, Brushfield spots, large ears with a pit on the right, short neck with redundant skin, bilateral simian creases, and fifth finger clinodactyly. She was wheelchair bound, had cortical blindness, bilateral profound hearing loss, severe speech delay and myoclonic seizures. Repeat chromosome analysis on peripheral lymphocytes showed 46,X,+mar[14]/47,XX,+21[12]/45,X[4]. FISH analysis revealed the marker was a ring X and lacked the X inactivation locus (XIST). At least three possible mechanisms could result in the observed cell lines. The first is that the original cell line was 47,XX,+21 with loss of the 21 giving a 46,XX cell line; a subsequent break in the X chromosome results in the 46,X,r(X) cell line. As ring chromosomes are unstable, they may be lost creating the 45,X cell line. A 46,XX cell line was not seen in this analysis, but its presence cannot be completely ruled out; it may be confined to a specific tissue. The second mechanism is a possible re-absorbed twin, one with a 47,XX,+21 karyotype and the other with a 46,X,r(X) and subsequent loss of the ring X. The third possibility is that this is a true chimera. The phenotype in cases with a ring X depends on the presence of the XIST locus and euchromatic material. If gene-coding material is present, in the absence of the XIST locus, it will be expressed, along with genes on the normal X chromosome. It results in a lack of dosage compensation for the X-linked genes and leads to an abnormal phenotype that may include mental and growth retardation and multiple congenital anomalies. The phenotype suggests the predominant cell line in this child is 46,X,r(X). The amount of euchromatic material that might be present on the ring X could not be determined.

Mild phenotype associated with tandem duplication of chromosome 16q. *L. Mehta*¹, *M.J. Macera*², *S. Sastry*¹, *S. Kleyman*², *A. Babu*². 1) Div. Medical Genetics, Schneider Children's Hosp./ North Shore Univ. Hosp., Manhasset, NY; 2) Div. Mol. Medicine & Genetics, Wyckoff Heights Medical Center, Brooklyn, NY.

A 15 year old Korean male was evaluated for mild mental retardation and behavioral abnormalities. As an infant he had a small cardiac septal defect that closed spontaneously and inguinal hernia repair. Growth was normal. Dysmorphic features were mild and included peaked anterior hairline, flat occiput, wide and flat nasal bridge, small ears with overfolded helices, short fifth fingers and widely separated toes. IQ was measured as 70. He had adequate verbal skills. Attention span was poor with hyperactivity and an immature affect. Plasma amino acids, urine organic acids and fragile X DNA studies were normal. Chromosome analysis of peripheral blood lymphocytes revealed an abnormal chromosome 16 with additional material in the long arm. Other chromosomes were normal. Parental karyotypes were normal indicating a de novo origin of the abnormal chromosome 16. FISH studies using whole chromosome paint 16 indicated that the additional material was derived from 16. Comparing band on band homology, bands 16q21-q22 were considered to be duplicated making the patient trisomic for 16q21-22. The karyotype was designated 46,XY,add(16)(q21).ish der(16)dup(16)(q21q22)(wcp16+). Direct interstitial duplications of chromosome 16 are rare but have been reported for 16p and proximal 16q segments. This is the first case report of direct duplication of 16q21-22. Only 5 reports of direct 16q duplications were ascertained; three were dup16q11-q13, one was dup16q13-q21 and one was dup16q11-q22. All 5 patients had moderate to severe mental retardation with inconsistent dysmorphic features. Our patient represents the smallest 16q duplication to date and has a significantly milder phenotype with only minor dysmorphisms and a clinically insignificant cardiac defect. Behavioral abnormalities are however reported in at least three of the other patients and were noted in our patient. Phenotypic correlations for partial trisomy 16 are difficult given the almost invariable association with other chromosome imbalance. Direct duplications such as this may yield more specific information in the future.

Analysis of dicentric Y chromosome in patient with Turner syndrome: clinical, cytogenetic, and molecular studies. *J.W. Kim¹, J.M. Kim¹, Y.M. Kim¹, I.O. Song², H.M. Ryu^{1,2}, Y.H. Cho³, S.Y. Park¹.* 1) Genetic Research Laboratory, Samsung Cheil Hosp, Seoul, Korea; 2) Department of Obstetrics and Gynecology, Samsung Cheil Hosp. Seoul, Korea; 3) Department of Medical Genetics, College of Medicine, Hyanyang University, Seoul, Korea.

We describe the results of clinical, cytogenetic and molecular studies of dicentric mosaic marker derived from chromosome Y. The patient referred for genetic study because of Turner syndrome with primary amenorrhea and has detected an abnormal karyotype of $mos\ 45,X[106]/46,X,+mar[27]$. We used special cytogenetic techniques, fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR) to characterize the structure and origin of the marker chromosome. The marker chromosome was confirmed negative NOR and faintly positive with C-, DA-DAPI banding. FISH using a LSI SRY/CEP X dual color probe analyzed the duplication copies of SRY gene and one copy of centromeric region in X chromosome as control. CEP Y(a)/CEP Y(satIII) dual color probe was indicated the duplication copies of centromeric region on Y chromosome. PCR analysis was shown to the presence between SRY and sY134 loci without sY156, sY254, sY255 and heterochromatin regions. PCR and FISH analysis confirmed to present two copies of the SRY gene and centromeric region in all analyzed cells. These are attractive tool for the character of the mosaic marker chromosome origin. Our results support the hypothesis that the coexisting 45,X cell line is more influential on the determination of the sex phenotype in patients with 45,X/46,X,dic(Y) mosaicism.

Down syndrome cerebellar phenotypes in Ts65Dn and Ts1Cje segmentally trisomic mice: Implications for the mechanism of gene action. *L.E. Olson¹, L.L. Baxter¹, E.J. Carlson², C.J. Epstein², R.H. Reeves¹*. 1) Johns Hopkins University School of Medicine, Baltimore, MD; 2) University of California at San Francisco.

In rare individuals with chromosomal translocations, Down syndrome (DS) is caused by partial trisomy of chromosome (Chr) 21. Correlation of the smallest regions of overlap in such individuals also displaying the same characteristics of DS has led to the hypothesis that many aspects of the phenotype arise from dosage imbalance of one or a few genes. An alternative hypothesis asserts that DS phenotypes result from small effects of hundreds of genes at dosage imbalance. Segmentally trisomic mice, which produce dosage imbalance for different numbers of Chr 21 gene orthologs, provide sensitive models in which to test this hypothesis.

By comparing mouse Chr 16 and human Chr 21, we know that Ts1Cje and Ts65Dn mice are respectively trisomic for 85 and 124 of the 225 catalogued Chr 21 genes. Ts65Dn mice, like individuals with DS, have reduced volume and cellular density in the cerebellum (Baxter et al., 2000); thus, trisomy for roughly half of the genes on Chr 21 is sufficient to produce this DS phenotype. The cerebellum of the Ts1Cje mouse is affected more mildly than that of the Ts65Dn mouse. Although cerebellar volume is reduced to the same extent as Ts65Dn mice (88% of euploid), granule cell density is substantially less affected in Ts1Cje (90% of euploid) as compared to Ts65Dn (76%). The Ts1Cje mice did not show the reduced Purkinje cell density found in Ts65Dn. This graded severity of the cerebellar phenotype with decreasing amounts of trisomy supports the hypothesis that multiple genes contribute to this phenotype of DS.

Baxter, L. L., T. H. Moran, J. T. Richtsmeier, J. Troncoso, and R. H. Reeves. 2000. Discovery and genetic localization of Down syndrome cerebellar phenotypes using the Ts65Dn mouse. *Hum Mol Genet* 9: 195-202.

Further clinical delineation and microsatellite profile in 1p36 deletion patients. *F.R. Vargas^{1,2,4}, J.C. Llerena Jr³, M.C. Ramos⁴, J.G. Barbosa-Neto⁴, K.R.L. Souza^{1,2}, H. Ramos³, R.R. Santos⁵, J.C. Cabral de Almeida³.* 1) Genetics, Instituto Nacional do Cancer, Rio de Janeiro, RJ, Brazil; 2) Universidade do Rio de Janeiro (UNI-RIO), Rio de Janeiro, Brazil; 3) Instituto Fernandes Figueira, FIOCRUZ, Rio de Janeiro, Brazil; 4) Gen Genetica Medica & Citogenetica Ltda, Rio de Janeiro, Brazil; 5) Instituto Estadual de Diabetes e Endocrinologia, Rio de Janeiro, Brazil.

The 1p36 deletion has been recently recognized as an apparently new chromosomal syndrome. We studied six unrelated cases of de novo 1p36 deletions. All of them showed only one signal with the fluorescence in situ hybridization (FISH) probe located at 1p36. In one of the patients the deletion was not visible in 550-800 GTG banded preparations. All six patients showed very similar clinical findings, allowing for the clinical recognition of this recently identified chromosomal syndrome. Approximately 30-40 cases of de novo 1p36 deletion have been identified to date (Am J Hum Genet 61:642-650, 1997; J Med Genet 36:657-663, 1999). The deleted chromosome appears to be of maternal origin in of cases, with at least three preferential breakpoint regions within 1p36. However, genotype-phenotype correlation does not seem to be straightforward. In regard to growth pattern, there seems to be two different phenotypes. While most affected children show postnatal growth retardation, some patients develop hyperphagia and obesity resembling Prader-Willi syndrome (PWS). Two of our patients fell in this category. Besides, two of our patients presented with dental anomalies that were not previously observed, which should probably be added to the clinical spectrum. Studies with a set of four microsatellites located at 1p36 (D1S243, D1S244, D1S450, D1S468) showed that the deleted chromosome was of paternal origin in at least two cases. Further investigation with a larger set of microsatellite markers spanning the 1p36 region is currently underway.

Thyroid Dysfunction, In children and adolescents with Down'Syndrome In Kuwait. *A.A. Sadika¹, R.L. Al-Naggar¹, N.M Al-Awadi², M.M. Abu-Henedi¹, L.A Bastaki¹.* 1) Obstetric, Kuwait Medical Genetic Center, Kuwait, Kuwait(KMGC); 2) West Salmiya health Clinic, Ministry of Health, Kuwait.

Objectives: The association of thyroid dysfunction with Down's syndrome(DS) has become well recognized, and abnormal thyroid function has been reported at all ages. The aim of this study is to evaluate the thyroid function in individuals with DS who are receiving health care in KMGC and related clinics. Methods: 61 patients and 40 normal controls were selected to be age and sex matched. Karyotyping was carried out to confirm diagnosis of DS and examined for evidence of hormonal and autoimmune thyroid dysfunction at the time of their referral for routine health care. Thyroxine(T₄), thyrotropine(TSH) and thyroid autoantibodies were measured by radioimmunoassay and hemagglutination tests respectively. Results: 15% of DS patients had hypothyroidism, 1.6% hyperthyroidism, 39% subclinical thyroid dysfunction and 44.4% of DS patients had euthyroidism. All hypothyroid patients had raised antithyroglobulin(ATG) and antiperoxidase(AP) antibodies. Elevated ATG antibody titre has been found in 25% of subclinical hypothyroid subjects. Thyroid dysfunction and autoantibodies have been found more at the ages of 10 to 20 years and the dysfunction is equally distributed by sex. Conclusion: The frequency of hypothyroidism in DS patients was in keeping with the other studies, while the frequency of subclinical hypothyroidism exceeded that reported in certain series. On the other hand the frequency of hyperthyroidism in DS patients was similar to that reported in the normal population. The positive thyroid autoantibodies in DS patients could be a possible cause of thyroid dysfunction despite of the insignificant association with age in our work. We recommend regular thyroid function assessment annually and every 6 months in case of positive thyroid autoantibodies. Down syndrome patients with any thyroid dysfunction should receive the proper management.

Paternally derived inv dup(15) chromosome associated with Prader-Willi syndrome. *D.J. Waggoner¹, R. Brown¹, S. Pihl¹, J. Kobori², S. Christian¹, D. Ledbetter¹.* 1) University of Chicago, Chicago, IL; 2) Permanente Medical Group, California.

Supernumerary marker chromosomes occur relatively frequently in the population with an incidence of 0.7-1.5/1000 live births. Inv dup(15) is the most common and accounts for approximately 50% of all supernumerary markers. Generally, inv dup(15) chromosomes can be classified into two groups, those containing the Prader-Willi (PWS)/Angelman syndrome (AS) critical region (large markers) and those not containing the PWS/AS critical region (small markers). Small inv dup(15) are commonly inherited and are not associated with a specific phenotype, although there is an increased incidence of uniparental disomy (UPD), which is usually maternal UPD and associated with PWS. Large inv dup(15) are almost always maternally derived and associated with a severe phenotype of seizures, mental retardation, dysmorphic features, and autistic behaviors. We describe four patients with paternal inheritance of a large inv dup(15) chromosome and maternal UPD, which represents a novel mechanism leading to PWS. Methylation studies show biparental methylation patterns, and microsatellite data confirms the paternal origin of these cases. The clinical phenotype of these patients is reviewed and reveals that despite the presence of one or two paternal copies of the PWS/AS critical region and normal methylation studies, there is no difference in the phenotype expected with PWS. The literature was reviewed and revealed on other case of paternally inherited large inv dup(15), with maternal UPD, Prader-Willi phenotype, and normal methylation studies. Mechanisms to explain these observations and a suggested approach for the evaluation of an inv dup(15) chromosome are explored.

Somatic Mosaicism in Sturge Weber Syndrome. *F.J. Serajee, M. Mally, D.C. Chugani, AHMM. Huq.* Departments of Pediatrics and Neurology, Wayne State University, Detroit, MI.

We have tested the hypothesis that lesions in the Sturge-Weber syndrome (SWS) result from somatic mutations in affected areas. Possible categories of such mutations include chromosomal anomalies and point mutations, deletions or duplications in a single gene. We collected samples from affected and unaffected tissues in six subjects with SWS and established short-term cultures. The cultured cells were then examined for chromosomal rearrangements. Cells cultured from unaffected areas revealed a normal chromosome pattern with expected random loss of chromosomes in all cell lines. However, short-term culture established from tissues from affected areas of two subjects revealed a significant proportion of cells with chromosomal abnormalities. In one subject, paracentric inversion of chromosome 4q (q22>q28) was present in 40% of cells cultured from leptomeningeal angiomas. The inversion was not present in cells cultured from blood of the same subject. Cells cultured from a port-wine stain of a second subject revealed trisomy of chromosome 10 in 50% of the cells. These abnormalities were not present in the blood or normal skin. Our study demonstrates that tissues from affected areas were genetically mosaic in selected patients with SWS, suggesting that mutant cells form a large subset of all cells in affected tissue. The lesions in SWS may result from somatic cell mosaicism for a disease causing chromosomal change or mutation. The chromosomal changes may also indicate chromosomal or genomic instability in affected areas in SWS. Alternatively, SWS lesions may simply be a marker of chromosomal mosaicism as in hypomelanosis of Ito.

Oral Motor, Speech and Voice Functions in Smith Magenis Syndrome Children: A Research Update. B.I.

Solomon¹, L. McCullagh², D. Krasnewich³, A.C.M. Smith³ and The SMS Research Unit. 1) Rehabilitation Medicine Dept., W.G. Magnuson Clinical Center, National Institutes of Health, Bethesda, MD; 2) W. G. Magnuson Clinical Center, National Institutes of Health, Bethesda, MD; 3) Medical Genetics, Branch, National Human Genome Research Institute, National Institutes of Health.

Smith Magenis Syndrome (SMS) is a known multiple congenital anomaly and mental retardation disorder associated with del of 17p11.2. The distinct clinical phenotype of developmental delays in speech/language, fine motor and gross motor skills as well physical and neurobehavioral features have been reported. Since our initial report (Sonies et. al, AJHG 61(4):A5), we have accrued 13 additional SMS children for study of oral motor and voice functions in the SMS population. We now report the findings of 27 SMS children (17F; 10M) ranging from 4 months-16 years. The presence of oral sensory motor deficits was seen in all of the children and included: oral tactile defensiveness, labial weakness, drooling, exaggerated lingual papillae, and open mouth posturing. Deficits in tongue range of motion and strength were seen in 26/27 of the children. Structural and physiologic anomalies of the larynx and hard/soft palates were seen in 21/25. Speech, at the time of evaluations, demonstrated various vehicles for expression including vocalizations, babbling and use of gestures and signs. Of the speaking children, velopharyngeal insufficiency and a perceived nasal speech quality was evident in 8/13 and voice impairments in 16/19. The impairments were characterized as hoarse, wet and harsh vocal qualities. Our findings are consistent with previously reported otolaryngologic reports in SMS children (Greenberg et. al, AJMG 62:247-254 and Sonies et. al, AJHG 61(4):A5) and do offer physiological explanations for the functional impairments seen in swallowing, speech and voice production. The clinical significance of the findings and its implications for speech and swallowing development and use of total communication as a treatment will be discussed.

Clinical delineation of chromosome 3q27>ter deletion: Rare aberration, variable phenotype. *H. Shapira¹, E. Shachar², A. Ramadan³, C. Halabi⁴, N. Kfir¹, H. Barel⁵, H. Dar⁵, T.C. Falik-Zaccai¹.* 1) Div. of Human Genetics, Hosp. for Western Galilee, Naharia, Israel; 2) Div. of Ped. Neurology, Rambam Med. Cent., Haifa; 3) Kupat Cholim Leumit, Nazereth; 4) Sherutei Briut Clalit, Haifa; 5) Cytogenetic Laboratory, Bnai Zion Med. Cent., Haifa, Israel.

A distal deletion of chromosome 3q is a rare aberration with only five previous reports in the literature. We report 2 new cases and summarize the clinical characteristics of this deletion. The first 2 years old girl presented with failure to thrive (FTT), microcephaly, down slanted eyes, absent tearing, hypertrophy of gingiva, short neck, hyperpigmentation of skin, excessive sweating, hypertonicity of four limbs and mild developmental delay. The second 4 years old boy presented with microcephaly, deep set eyes, epicanthal folds, strabismus, prominent nasal bridge and nose, high arched palate with velopharyngeal incompetence, small ears, short neck, mild ataxia, bilateral inguinal hernia and mild development and speech delay. Both children had an abnormal karyotype 46,XX and 46,XY respectively with del 3q27>ter. Three females and 2 males were previously described with this deletion. In the first case mild dysmorphic features and closed parietal meningocele were found along with inability to walk at the age of 20 months, and increased tone in the lower limbs. Dilatation of sweat glands and a mild skin rash were determined as well. Another 8 years old boy is described with short stature, FTT, small ears and high arched palate, hypotonia and mild developmental and speech delay. In 3 other cases bilateral anophthalmia/ micro-ophthalmia, congenital heart defects, abnormal genitalia, micrognathia, small ears, short nose, short neck and microcephaly were described. Deletion of 3q27>ter presents with variable phenotypes. Each additional report is of significance for its clinical delineation. Developmental delay, abnormalities of muscular tone, small ears, short neck, broad nose, FTT, and microcephaly appear to be the rule. Severe congenital anomalies in different organs are common. Differences in the molecular boundaries and parental origin of the deletion might be possible explanations for the variable phenotypes.

Unusual phenotype and cytogenetic features in a case of trisomy 18 due to isochromosome 18q: clinical, cytogenetic and molecular characterization. *T. Sahoo*¹, *R. Naeem*¹, *K. Pham*¹, *S. Chheng*¹, *S. Noblin*², *C. Bacino*¹, *M.J. Gambello*³. 1) Dept Human & Molec Gen, Baylor Col Medicine, Houston, TX; 2) Ob & Gyn and Genetics, LBJ General Hospital, Houston, TX; 3) Pediatrics and Medical Genetics, UTHSC Houston, Medical School, Houston, TX.

Trisomy 18 is a recognizable syndrome with distinctive clinical features. Unlike full trisomy 18, trisomy due to isochromosome 18 [i(18)(q10) or, i(18)(p10)] has been recognized to present with diverse phenotypic features. We report the characterization and genetic studies in a patient with an unusual presentation of trisomy 18 syndrome due to an isochromosome of 18q with partial monosomy for 18p. The proband was a male child born at 33 weeks gestation to a 24 year old mother with poor antenatal care and a complicated neonatal course. The infant presented with features of trisomy 18, including: intrauterine growth retardation, simple low-set ears, micropenis, cardiovascular defects and bilateral absence of radii and thumbs. Additional clinical features included: brachycephaly, median cleft lip and palate, hypotelorism, mid-facial hypoplasia and transient thrombocytopenia early in the neonatal period. Differential diagnoses at initial examination included chromosomal abnormalities, thrombocytopenia-absent radii syndrome (TAR), Roberts syndrome and Fanconi anemia. G-banded cytogenetic analysis revealed replacement of one chromosome 18 by an isochromosome for the long arm of chromosome 18 [46,XY,i(18)(q10)] in all cells. Additionally, 70% of the cells showed a derivative chromosome 10 that was shown by FISH analysis to arise from an unbalanced translocation between 18pter and 10qter. Interestingly, the derivative chromosome 10 retained its q-arm telomere in addition to the translocated 18qtel. The isochromosome 18q was monocentric as seen by C-banding and FISH for chromosome 18 centromere. Parental karyotypes were normal. The unusual phenotype in this infant was, therefore, due to trisomy for 18q and partial monosomy for 18p. Additional cytogenetic and molecular studies to elucidate the mechanism of formation of isochromosome 18q and retention of dual telomeres at 10qter in this case will be important.

A case of severe developmental delay with pharyngeal anomaly due to a *de novo* translocation [46, XY, t(6;12)(q16;p12)]. K. Yamada¹, T. Ono¹, T. Ohki², Y. Yamada¹, N. Ishihara^{1,3}, K. Miura², T. Kumagai², S. Sonta¹, N. Wakamatsu¹. 1) Dept Genet, Inst Developmental Res; 2) Pediatric Neurology, Central Hosp, Aichi Human Serv Ctr, Aichi; 3) Dept Pediatr, Nagoya Univ Sch Med, Nagoya, Japan.

We report a case of a four-year-old boy with a *de novo* translocation t(6;12)(q16-21;p12), severe developmental delay and a pharyngeal anomaly. He suffered profound mental retardation and severe delayed motor development with repeated bouts of aspiration pneumonia. Fluorescent *in situ* hybridization (FISH) experiments were performed to determine the break points of each chromosome using RP11-BAC clones. The results showed that in chromosome 6q16-21 to be located within a single BAC clone of 98I9 and that of chromosome 12p12 between 684O24 and 841C19. We performed Southern analysis using *MGC23817*, *COQ3* and *DKFZp564B0769* cDNAs of 6q16-21 and a *PEPP2* cDNA of 12p12 as probes. Although all these genes are included in the above BAC clones, none demonstrated any breakpoint. More detailed studies including analysis of the promoter regions of each gene are necessary to determine the molecular mechanisms underlying this disease.

Diminished plasma levels of zinc and alterations in lymphocyte subpopulations in Down syndrome patients. *M.L. SOTO QUINTANA¹, F.A. ALVAREZ NAVA¹, A. ROJAS ATENCIO¹, V. GRANADILLO¹, D. FERNANDEZ¹, A. OCANDO¹, E. LOPEZ¹, W. FULCADO².* 1) UNIDAD DE GENETICA MEDICA, UNIVERSIDAD DEL ZULIA, MARACAIBO, ZULIA, VENEZUELA; 2) HOSPITAL CLNICO DE MARACAIBO.

Alterations of plasma levels of zinc and immune system in Down syndrome (DS) had been reported. These alterations had been associated with high infectious illness rate, which represents the main cause of mortality in this disease. The objectives of this study are to determine plasma levels of zinc and to evaluate the immune system in DS patients. Peripheral blood samples from 43 DS patients were extracted. These patients were examined at Unidad de Genetica Mdica, Universidad del Zulia and their mean DS of age were 2,3 2,0 years. As control group 40 healthy children were studied (mean DS 2,3 2,0 years). Karyotypes by standard technique, determination of plasma levels zinc by absorption atomic spectrofotometry and evaluation of immune system by flow cytometry were carried out in study group. All DS patients were free trisomy 21. Significant diminished plasma levels zinc, helper T lymphocyte (CD4) percentage, helper/cytotoxic (CD4/CD8) ratio and B- cells (CD19) were found in DS patients by matching with control group. No significative difference between DS patients with diminished plasma levels zinc and DS patients with normal zinc were found. These findings suggest that zinc deficiency is not sole etiology involved in disorders of immune system seen in DS patients. Other factors, such as thymic alterations, molecular abnormalities due to gene overexpression of loci located on chromosome 21 could be involved. To supply according to needed dosage in DS patients with zinc deficiency is recommended to adjust the disturbance that this deficiency might provoke in these patients. Thus, morbidity-mortality indexes could be diminished in this disease.

Deletion for chromosome 21 with abnormal brain morphogenesis. *G. Yao¹, X-N. Chen¹, R.P. Morse², J.R. Korenberg¹.* 1) Med Gen, Cedars-Sinai Med Ctr, UCLA, Los Angeles, CA; 2) Dartmouth-Hitchcock Med Ctr, Lebanon, NH.

Monosomy 21 is usually lethal prenatally. Therefore to identify the genes responsible, we have previously defined the molecular structure and clinical features of a panel of 14 patients with independent partial deletions of chromosome 21. Here we report a new deletion 21 (HH) and its molecular analysis to further explore the correlations between brain phenotype and genotype. The subject, HH, is a 2 year old boy with global developmental delay and intractable, epileptic seizures. There is facial asymmetry with left ocular buphthalmia and right microphthalmia accompanied by bilateral lenticular fibrosis. He also has microcephaly, a broad nasal bridge, micrognathia, unilateral hearing loss, moderate pes planus and forefoot abduction, bilateral inguinal hernia, and micropenis. MRI of the brain revealed a paucity of cerebral white matter, most marked in the left parietal lobe, diffuse ventriculomegaly and widening of the sulci, hypoplastic cerebellar vermis and corpus callosum, symmetrically dysplastic hippocampus, and decreased subcortical white matter in the frontal and temporal lobes and insula, consistent with delayed myelination. The pathologic report for globe shows proliferating fibroblasts which extend through the pupil and surround the iris. Small atrial septal defect (ASD) was noted and abnormally thick sternum detected on X-ray, and the sternum and pelvic bones appears sclerotic for newborns. Cytogenetic analysis revealed 46, XY,del(21)(q22.1). Molecular analysis employed Fluorescence in situ Hybridization (FISH) using BACs and Cosmids spanning the region from D21S232 (21q11.1) to D21S403 (21q22.3 distal), representing twenty chromosome 21 markers. We determined that the deletion encompassed genes/markers from ETS2 through D21S403 (LA 161) and included the genes for ETS2, DSCAM(D21S348 D21S349), PFKL, ITGB2 and COL 6A1. Our current case provides evidence that the genes in the region of 21q22.3 are involved with the morphogenesis of the brain and may help to unravel the genetic origin of the cognitive defects of Down syndrome.

Contiguous gene deletion syndrome involving the TWIST locus and the HOXA cluster. *R. Kosaki¹, Y. Yoshida², H. Ohashi¹, T. Takahashi², K. Kosaki².* 1) Div Medical Genetics, Saitama Children's Medical Ctr, Saitama, Japan; 2) Dept Pediatrics, Keio Univ Sch Med, Tokyo, Japan.

Haploinsufficiency of the TWIST gene at 7p21 is associated with the Saethre-Chotzen syndrome characterized by craniosynostosis, maxillary hypoplasia, prominent ear crus, anal atresia, and short fifth fingers. Haploinsufficiency of the HOXA13 gene at 7p15 leads to the hand-foot-genital syndrome characterized by postaxial limb defect and anogenital defects. The phenotype of the deletion of the entire HOXA gene resembles that of mutations in HOXA13, most likely due to functional redundancy of the remaining HOX genes. We present a patient with 46,XY,del(7)(p15.3p21) who had combined features of Saethre-Chotzen syndrome and hand-foot-genital syndrome. The patient was a Japanese newborn male. Characteristic features were flat supra-orbital ridges, proptotic eyes, prominent ear crus, short fifth fingers, anal atresia with rectal dermal fistula. FISH analysis revealed deletion of TWIST and the entire HOXA cluster. We thus conclude that the combination of Saethre-Chotzen syndrome phenotype, postaxial limb defects, and the anal defect in this patient resulted from combined haploinsufficiency of TWIST and the HOXA cluster. Hence, the phenotype may define a new contiguous gene syndrome on 7p.

PMX2B, a new candidate gene for Hirschsprung disease. *H. Karmous, B. Laudier, JM. Lapierre, D. Sanlaville, S. Lyonnet, T. Attie, M. Vekemans, MC. de Blois, J. Amiel, S.P. Romana.* Departement de Genetique, Hopital Necker-Enfants Malades, Paris, France.

Hirschsprung disease (HSCR) is a congenital intestinal malformation of the enteric nervous system. It is a multigenic malformation and until now, 8 genes have been involved in the etiology of this disease: genes encoding proteins of the RET signaling pathway (RET, GDNF and NTN), genes participating in the endothelin (EDN) type B receptor pathway (EDNRB, EDN3 and ECE), the SOX10 gene and, the SIP1 gene that is mutated in syndromic forms of HSCR. However alterations of these genes are found in not more than 50-60% of affected individuals. We report here the results of a molecular cytogenetic study that identifies the PMX2B gene as a new candidate gene for HSCR disease. The patient is a 2-year-old girl who presents a short segment HSCR, facial dysmorphism and developmental delay. High resolution karyotype detected a t(4;8)(p13;p22) translocation with a 4p13 deletion confirmed by CGH. A molecular characterization of this rearrangement was undertaken using a series of specific 4p and 8p probes. The 8p22 breakpoint maps between BAC 279L11 and BAC 143D15, which are 3Mb apart. No compelling HSCR candidate gene for HSCR was localized in this region. The 4p13 deletion maps between BAC 1102H24 and 1147H2, a region of 5Mb in length. Interestingly, in this region lies the Paired Mesoderm Paired-type Homeobox Gene (PMX2B) (MIM 603851), a gene involved in the RET signaling pathway. We confirmed PMX2B gene deletion in our patient using FISH with BAC 227F19 encompassing the PMX2B locus. PMX2B is essential for the development of autonomic neural crest derivatives. It has been demonstrated that the defect in the enteric nervous system of *Pmx2b*^{-/-} mice at E10.5 is similar to the one observed in *Ret*^{-/-} mutants. Moreover, *Ret* expression is absent in the sympathetic and enteric ganglia of *Pmx2b*^{-/-} mice. Finally, we analyzed the expression pattern of PMX2B during human embryonic development that shows an intense PMX2B expression in the gut. So far, the PMX2B gene has not been implicated in HSCR. The present observation suggests however that PMX2B haploinsufficiency might predispose to HSCR.

Hemizyosity of d-Catenin (CTNND2) and Semaphorin F genes is not always associated with severe mental retardation in Cri-du-chat syndrome. A. Salin, C. Ozilou, O. Raoul, MC. de Blois, M. Prieur, A. Munnich, M. Vekemans, C. Turleau, S.P. Romana. Departement de Genetique, Hopital Necker-Enfants Malades, Paris , France.

It has been proposed that the critical region for mental retardation of the Cri-du-chat syndrome (CdCCR) was lying in 5p15.2 band. However, a controversy still exists about the precise location of the CdCCR in the 5p15.2 band. A first region have been proposed with the Semaphorin F as a candidate gene and a second region, more proximal, with the d-Catenin as a candidate gene. We report here the molecular cytogenetic characterisation of 3 patients (two with interstitial deletion) with del(5p) detected by high resolution banding and without severe mental retardation. Because of the atypical features of these patients, we decided to map their deletion using FISH with YACs encompassing 5p14-p15 bands. We found that patient 1 had a terminal deletion with a proximal bin between YAC 938G6 (deleted) and YAC 747B11 (present) attesting a terminal del(5)(p15.2) encompassing the two proposed CdCCR. The second and the third patients have both an interstitial deletion mapped between YAC938G (present) and YAC 933G1, split for patient 2 and present for patient 3. These data attested for these two patients an interstitial del(5)(p15.1p15.1) encompassing only the most proximal CdCCR proposed region. Because the Semaphorin F and the d-Catenin are candidate genes for the severe mental retardation of the Cri-du-chat syndrome, we searched for haploinsufficiency of these two genes using FISH with BAC/PAC clones 502L6, 162O17, 248B21 encompassing the d-Catenin gene and BAC 5H13 clone encompassing the Semaphorin F gene. We found that the Semaphorin F was deleted in patient 1 (terminal deletion) and present for the two other patients. The d-Catenin gene was deleted in patient 1, split in patient 2 and present in patient 3. Altogether, these data indicate haploinsufficiency of the Semaphorin F and d-Catenin are not always linked to the severe mental retardation of the Cri-du-chat syndrome.

"Phylloid" pattern skin mosaicism in a 15 month old girl with multiple congenital malformations and mosaicism for partial monosomy13 [46XX/46XX,-13,+r(13)]. *F.A. Collins¹, K. Maclean¹, Z.H. Wu², P. Malafiej², A.*

Darmanian², A. Daniel², G. Peters². 1) Dept Clinical Genetics, Children's Hosp at Westmead, Sydney, Australia; 2) Dept Cytogenetics, Children's Hospital at Westmead, Sydney Australia.

A 15 monthold girl was referred for evaluation of a rapidly progressive scoliosis. She presented with severe retardation and multiple congenital anomalies. Her skin showed streaky hypo- and hyperpigmentation following along the lines of Blaschko over all her limbs and thorax. In addition there were irregular, variable sized oval patches of hyperpigmentation over trunk and limbs.

Blood karyotype was 46XX, in all cells analysed, Skin biopsies were taken from two separate sites - one apparently hyperpigmented and one hypopigmented. Karyotype analysis showed predominantly abnormal karyotype: 46XX,-13,+r(13), in 95% of cells. FISH studies confirmed that the fragment was a small ring 13, retaining only the pericentric component of 13q.

Mosaic skin pigmentary disorders following the lines of Blaschko have been reported with X chromosome abnormalities as well as other autosomal chromosomal mosaicism. This patients's skin is suggestive of a "phylloid" pattern as described by Happle. The phylloid pattern has predominantly been reported in cases with trisomy 13 mosaicism. This patient is of interest because of the resulting partial monosomy 13.

X inactivation studies are presented from blood and skin and show non random X-inactivation in the fibroblast cell line suggesting the abnormal cell line originated in a somatic cell, subsequent to X inactivation.

The association of chromosome 13 trisomy and/or deletion with phylloid pigmentary mosaicism may be a chance event. However there are a few potential genes on chromosome 13 which may explain its involvement. The potential role of Endothelin type B receptor (EDNRB) located at 13q22 and Dopachrome tautomerase (DCT) are discussed.

Ring chromosome 9: clinical and cytogenetics characterization. *D. Concolino¹, M.T Moricca¹, M.A. Iembo¹, R. Apa¹, D. Caloiero², E. Saullo², P. Strisciuglio¹.* 1) Dept.of Pediatrics, University Magna Graecia Catanzaro, Catanzaro Italy; 2) Div.of Pediatrics Soveria Mannelli Hospital, Italy.

The syndrome associated to ring chromosome 9 is not commonly observed and phenotypic manifestations may be extremely mild, particularly in the neonatal period. We report a female infant with ring 9 mosaicism detected by routine cytogenetic analysis and characterized by FISH studies with specific sub-telomeric probes of chromosome 9 as del 9p (pter-p24). The proband was the second child of healthy and unrelated parents. The family history, the pregnancy and the delivery were unremarkable. Physical examination at the age of 1 month showed normal weight, length and head circumference, mild dysmorphic features, cleft soft palate. The echocardiography showed a mild ventricular septal defect. The neuropsychiatric examination at the age of 19 months showed moderate psychomotor retardation. Chromosome analysis was performed on QFQ banded metaphases from synchronized peripheral lymphocyte cultures using standard procedures. Proband's karyotype was interpreted as 46,XX(35%)/46,XX, r(9)(65%). The ring 9 was investigated by FISH with sub-telomeric probes for 9p and 9q. The 9q sub-telomeric region was intact but there was no 9p ter fluorescent signal on the ring chromosome 9 and in the chromosome 9 of normal lines. The FISH analysis with chromoprobe multiprobe-T system (Cytocell) in both parents resulted normal. These results suggest that in our patient is present a de novo del 9p syndrome with mild phenotype. Further molecular studies are necessary to determine the extent of the deletion in the 9 pter region in order to compare the phenotype of this patient with the size of telomeric deletions.

Partial trisomy 1q: A phenocopy of neonatal Marfan syndrome. *K.L. David¹, C.S. Cressman¹, P.A. Ankola², M.A. Perle³.* 1) Genetics/Dept. of Pediatrics, Metropolitan Hospital Center, NY, NY; 2) Neonatology/Dept. of Pediatrics, Metropolitan Hospital Center, NY, NY; 3) Dept. of Pathology, NYU School of Medicine, NY, NY.

Neonatal Marfan syndrome is a well-documented disorder presenting with arachnodactyly, flexion contractures, joint laxity, loose redundant skin, and severe cardiac valve insufficiency and dilation of the aorta. Mutations in the fibrillin 1 gene on chromosome 15 have been detected in this syndrome. We report a chromosomally abnormal infant whose peripheral blood G-banded karyotype revealed a de novo $46,X,del(X)(q22.3)/46,X,der(X)t(X;1)(q22.3;q21)$. This infant was initially suspected to have neonatal Marfan syndrome. The infant's features at birth included severe arachnodactyly of fingers and toes, loose skin, pectus carinatum, aplasia cutis congenita of the scalp, hyperextensible joints, and septal hypertrophy and ASD on echocardiography. While many features were suggestive of neonatal Marfan syndrome, aplasia cutis congenita has not been reported in that condition. On the neonatal exam, no features could be clearly ascribed to the $46,X,del(X)(q22.3)$ cell line. Review of the literature on partial trisomy 1q reveals a significant phenotypic overlap with neonatal Marfan syndrome. In addition, patches of skin aplasia have been noted in some cases of partial trisomy 1q. This cytogenetic entity should be considered in the differential diagnosis of neonatal Marfan syndrome. X chromosome inactivation studies are planned.

***De novo* interstitial deletion of chromosome 14q that helps further localize a putative gene for eye and CNS embryonic development.** *G.S. Kupchik¹, S.K. Barrett¹, M. Velinov^{1,2}, M. Macera³, A. Babu³, M. Genovese², S. Brooks², M. Strominger¹, E.C. Jenkins².* 1) Dept Pediatrics, Maimonides Medical Ctr, Brooklyn, NY; 2) Dept Cytogenetics, New York State Institute of Basic Research, Staten Island, NY; 3) Wyckoff Heights Medical Center, Brooklyn, NY.

A newborn male presented with IUGR and significant facial dysmorphic features. Chromosome analysis showed 46,XY, del(14)(q22.3q24.1), a *de novo* deletion of 14q. He had a VSD and duplex collecting system of the right kidney with absent left kidney. Brain MRI was significant for vermis hypoplasia, but the pituitary gland was present and normal. On physical exam, he had an unusual umbilicus, hypoplastic nails and the sandal sign. He twice failed ABR-based hearing evaluation. Ophthalmology exam showed a normally formed anterior segment. On dilated funduscopy, there were large central optic nerve pits. The neuroretinal rim was normal with mild peripapillary pigment hyperplasia. There was no evidence of iris, retinal or choroidal coloboma. The child is currently 18 months of age and has significant growth and developmental delay.

Two other cases with interstitial deletion involving band 14q22 were reported to date: deletion(14)(q22.1q22.3)(Elliot J et al., J. Med. Genet 20:251, 1993) and deletion(14)(q22q23)(Bennet CP et al, J. Med. Genet 28(4):280; 1991). In these two cases, the phenotype included anophthalmia and absent pituitary gland. Our patient does not have anophthalmia and the pituitary is present. Since the breakpoints in the present case are more distal than those reported in the previously published cases with anophthalmia and absent pituitary, it is likely this is due to deletion of chromosome material from the proximal 14q22 region. This region, therefore, could be a likely location for a gene(s) with an important role in eye and CNS development on chromosome 14.

Autosomal dominant inheritance of infantile myofibromatosis. *D.J. Zand¹, S. Saitta¹, K. Russell¹, D. Huff², E. Zackai¹.* 1) Division of Human and Molecular Genetics, Department of Pediatrics; 2) Department of Pathology, CHOP, Philadelphia, Pa.

We present two families with infantile myofibromatosis (IM) in multiple generations, and suggest that despite previous classification in OMIM (#228550) as autosomal recessive (AR), it is inherited in an autosomal dominant (AD) manner. In Family I, individuals were diagnosed by both clinical and pathological examination: a father (multiple progressive lesions since birth inclusive of meningeal, soft tissue, vocal cords, and kidney), his daughter (soft tissue and bone lesions present at birth), and his maternal nephew (multiple lesions since birth inclusive of bone soft tissue, polyposis of the GI tract). By history, the father's sister and their mother all had single nodules removed. In Family II, clinical examination and/or pathological review have been completed for 5 family members: a father and two male children, his maternal nephew and his nephew's male child, presenting with soft tissue and/or bone lesions at birth or within the first two years of age. By history, the father's sister and their father also had nodules removed. Review of the literature revealed an affected father and daughter (*Am J Surg Path* 8:529-538,1984), an affected grandfather, daughter and four grandchildren (*Schweiz Med Wschr* 101:1381-1385,1971), and two maternal half siblings (*Klinische Wochenschrift* 88:92-94,1976). Our families together with these support autosomal dominant inheritance. We suggest that pedigrees with only siblings affected may represent AD with non-penetrance or spontaneous regression of nodules in the "unaffected parent".

Features of Kabuki syndrome in a child with deletion 7p21ter. *P.D. Turnpenny¹, C. Hayes², R.P. Martin³, R.T. Howell⁴.* 1) Clinical Genetics, Royal Devon & Exeter Hosp, Exeter, UK; 2) Child Health, Royal Devon & Exeter Hospital, Exeter, UK; 3) Paediatric Cardiology, Royal Hospital for Sick Children, Bristol, UK; 4) Cytogenetics, Southmead Hospital, Bristol, UK.

More than 300 cases of Kabuki syndrome (MIM 147920) (KS) have been reported worldwide but the cause of the condition remains to be elucidated. The large majority of cases are sporadic with no increased incidence of parental consanguinity. There have been isolated cases reported in association with various chromosome aberrations on 1p, 4p, 6q, 12q, 13q, 15q, 17q and Xp. We describe a male patient with many features of KS, the first child of non-consanguineous Sudanese parents, with a de novo cytogenetically visible terminal deletion of chromosome 7p21, confirmed by sub-telomeric FISH probing. He was symmetrically growth retarded at full term after a normal pregnancy, birth weight 1.8 kg. He was diagnosed with Tetralogy of Fallot at 6 weeks and exhibited severe failure to thrive with feeding difficulties, gastro-oesophageal reflux and mid-gut malrotation. His dysmorphic features comprise unusual facies with interrupted eyebrows, marked epicanthic folds extending well below the lower eyelid, upslanting, long palpebral fissures, large ears, down-turned mouth, high palate, mild fetal finger pads, coronal hypospadias, and a sacral dimple. Cranial MRI scan showed some hypoplasia of the deep white matter and renal ultrasound was normal. Postnatal growth failure was persistent and severe and, although manifesting developmental delay, some neurodevelopmental progress has taken place. He has some features in common with other reported cases of 7p deletion but his striking peri-orbital 'Kabuki' features are not generally seen in these reports. KS may be a phenotype with significant genetic heterogeneity.

No association between dermatoglyphics and atopic asthma in children. *E. Corona-Rivera¹, J.R. Corona-Rivera^{1,2}, A. Santana-González², J.J. Pérez-Molina², A. Corona-Rivera¹, E. Nuñez-Nuñez².* 1) Laboratorio de Genética Humana, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, México; 2) División de Pediatría, O.P.D. Hospital Civil de Guadalajara Dr. Juan I. Menchaca.

Four previous reports inform the presence of dermatoglyphic anomalies in children with atopic asthma (AA), however, we were not able to identify common consistent patterns in such reports. We evaluated dermatoglyphic prints from 100 children with AA (45 girls/55 boys; mean age 9.9 SD 3.3ys), and their controls (39 girls/61 boys; mean age 10.1 SD 3.3ys). AA diagnosis was made according to the NHLBI criteria. Atopy was determined by prick skin test in 95 cases and by EAST test in five cases. Allergic rhinosinusitis and atopic dermatitis were present in 44% and 12% of the AA cases, respectively. Control group was integrated by 100 children without atopic problems. Previous parents consent, absence of atopy was confirmed by negative prick skin test. Ten quantitative dermatoglyphic variants were considered in both hands of each group (number of archs, whorls, ulnar loops, radial loops, a-b ridge count, total finger ridge count (TFRC), d-t ridge count, atd angle, tda angle and main-line index), as well as 30 additional qualitative patterns in which odds ratios and 95% CI were considered. Children with AA showed decreased average a-b ridge counts at each hand (right hand: 41.5 SD 5.4 vs. 43.3 SD 4.8, $P=0.01$; left hand: 42.2 SD 6.7 vs. 44.1 SD 5.5, $P=0.05$), and both hands (83.8 SD 11.1 vs. 87.4 SD 9.5, $P=0.02$). No other statistical differences were observed in the remaining quantitative and qualitative dermatoglyphic patterns. Distally displaced axial triradius, larger TFRC, increased whorls, decreased archs and low d-t ridge count, previously informed in children with AA, were not confirmed in this group. The lower a-b ridge count found was within normal reference studies range, and had limited clinical significance in reported entities with low a-b ridge count. We conclude that considered dermatoglyphic traits were not genetic indicators to the occurrence of AA in children.

A Puerto Rican boy with dislocated hips and radial heads and carpal coalition: a new case of a syndrome described only in Puerto Rican children. *E.S. Moran¹, S. Vepa², J.G. Pappas³.* 1) Orthopedic Inst, Hosp Joint Diseases, New York, NY; 2) Dept Pediatrics, New York Univ, Sch of Med, New York, NY; 3) Human Genetics Program, Dept Pediatrics, New York Univ, Sch of Med, New York, NY.

We describe a 12 year old boy who presented with multiple joint dislocations, limited range of motion of hips, knees and elbows and mild developmental delay. Clinical examination revealed height at the 10th percentile, upper to lower segment ratio equal to one, elongated face with malar hypoplasia, myopia, high arched palate, prognathism, bilateral dislocated elbows and patellae with limited range of motion of wrists, hips and knees. X-rays revealed dislocation of the radial heads, fusion between the lunate and triquetrum, markedly shallow acetabulum bilaterally, right greater than left, subluxed left femoral head and superior-lateral dislocation of the right femoral head. Shortened femoral necks and bilateral sclerotic lateral femoral condyles with small ossific fragments were present. Multiple vertebral anomalies of the cervical spine with concomitant atlantoaxial instability were present. He was status post surgical stabilization of C1-C2. By report, the proband's mother had congenital bilateral external rotation of feet and early onset myopia. Our case had a full sibling and a maternal half sibling; both female and unaffected. The features of our case are characteristic of a syndrome described in one publication of 23 Puerto Rican children (HH Steel et al, J Bone Joint Surg A 1993;75:259-264). Common features of our case and the published cases include dislocation of the hips, dislocated radial heads, short stature, fusion of the lunate and triquetrum and atlanto-axial subluxation. Our case had childhood onset myopia, elongated face, malar hypoplasia, high arched palate and mild developmental delay. The features of our case may expand the phenotype of the syndrome. In addition, there may be incomplete expression of the same syndrome in his mother which suggests autosomal dominant inheritance.

Clinical differences in subjects with Prader-Willi syndrome having type I or type II deletions. *N. Kibiryeve¹, D. Bittel¹, Z. Talebizadeh¹, T. Thompson², M.G. Butler¹.* 1) Childrens Mercy Hospitals and Clinics and University of Missouri-Kansas City School of Medicine, Kansas City, MO; 2) University of Kansas Medical Center, Kansas City, KS.

Prader-Willi syndrome (PWS) is due to the absence of paternal genes from the 15q11-q13 chromosome region. The major features of PWS include infantile hypotonia with feeding problems, mental deficiency and behavior problems, small hands and feet, hypogonadism and hyperphagia leading to marked obesity. The majority of subjects have an interstitial deletion of the 15q11-q13 region. Two proximal breakpoints (BP1 and BP2) have been reported centromeric to locus ZNF127. The second proximal breakpoint (BP2) lies between loci D15S541/S542 and D15S543 while breakpoint BP1 is proximal to D15S541/S542. Type I deletion is larger and involves BP1 while the type II deletion involves BP2 located about 500 kb distal to BP1. We analyzed clinical, anthropometric and behavioral data in 13 PWS subjects (6 males, 7 females; mean age 25.0 + 8.6y) with type I deletion and 12 PWS subjects (4 males, 8 females; mean age 18.8 + 5.9y) with type II deletion. We found significant differences between these two groups in multiple psychological tests. For example, PWS deletion type I subjects showed significantly worse self injurious behavior [e.g., externalized maladaptive index score (-14.1 + 14.6 for type I vs. -2.2 + 7.2 for type II: t-test; p=0.018)] compared with type II deletion subjects. Adaptive behavioral scores [e.g., motor skills (27.2 + 6.6 for type I vs. 42.4 + 13.6 for type II: t-test; p=0.002)] were significantly worse in PWS subjects with deletion type I. In addition, obsessive compulsive behavior [e.g., YBOCS control compulsion (3.2 + 1.5 for type I vs. 1.9 + 1.3 for type II: t-test; p=0.045)] was more evident in PWS subjects with deletion type I. PWS deletion type I subjects also had more difficulties with reading and mathematics and visual-motor integration. The results of this study indicate that the loss of genetic material between breakpoints BP1 and BP2 in the 15q11-q13 region significantly increases the severity of behavioral and psychological problems associated with Prader-Willi syndrome.

Kabuki syndrome with profound motor and intellectual disabilities. *H. Kawame*¹, *N. Fueki*², *Y. Fukushima*³. 1) Div. Medical Genetics; 2) Div. Neurology, Nagano Children's Hospital, Nagano, Japan; 3) Div. Clinical and Molecular Genetics, Shinshu Univ. Sch. Med., Nagano, Japan.

Kabuki syndrome (Niikawa-Kuroki syndrome) is a disorder of unknown cause with characteristic facies, postnatal growth retardation, mental retardation, and other anomalies. The phenotypic spectrum is very broad and a wide range of associated anomalies has been described. The range of motor and cognitive abilities is also broad, but most of patients in the literature were reported to have moderate or mild retardation. We present a 4-year-old female with profound motor and intellectual delay. She was born at 40 weeks gestation. Her birth weight was 2865 g, length, 50 cm. At birth, she was found to have coarctation of the aorta, VSD, and PFO. She also had imperforate anus with anovestibular fistula, and sacral dimple. Heart surgery was performed on 10 day life. After the surgery, she developed chronic lung disease due to recurrent airway infections and had tracheostomy at age 6 months. She had GERD, bilateral severe sensorineural hearing loss, and frequent otitis. Her MRI at age 24 months revealed delayed myelination and thinning of corpus callosum. On examination at age 4 years, her length was 85 cm (-4.0SD), weight; 10.8 Kg (-2.6SD), and OFC; 44.8 cm (-2.9SD). She continued to receive gavage feeding and tracheostomy. She rolled over, but lacked complete head control. She watched face and vocalized but had no meaningful words. She recognized her parent. She had characteristic facies and persistent finger pads. Along with common and more characteristic anomalies for Kabuki syndrome, such as coarctation of the aorta, and anovestibular fistula, she was diagnosed to having Kabuki syndrome. Her chromosome was normal, 46,XX. In the literature, only a few severely retarded and nonambulatory patients with Kabuki syndrome have been reported. We confirm that Kabuki syndrome should be considered as a differential diagnosis for patients with multiple congenital anomalies and severe motor and intellectual disabilities.

Cervical spine instability in patients with the 22q11.2 deletion. *E.H. Zackai, K. Russell, L. States, J. Tamai, M. Tonnesen, E. Simon, J. Catanzaro, P. Schiffman, D. Drummond, D.M. McDonald-McGinn.* The Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA.

A patient with a 22q11.2 deletion was found incidentally to have congenital cervical spine abnormalities (fusion posterior elements of C2-C3) during an evaluation to determine the presence of a laryngeal web. Following this, we retrospectively reviewed dental lateral cephalometric radiographs in 11 patients with 22q11.2 deletion and 6 (55%) had congenital cervical spine abnormalities: congenital fusion of C2-C3, an abnormally shaped dens, anomalous C1. Prompted by these findings, we prospectively obtained flexion-extension cervical spine radiographs in 39 consecutive patients with the 22q11.2 deletion 3 years of age and older, including the index patient. We noted abnormalities including an abnormally shaped dens in 77%, upswept C2 in 76%, hypoplastic/anomalous C1 in 59%, posterior element fusion with block vertebrae in 23% and without block vertebrae in 21%, and occipitalization of the atlas in 8%. Ten of the thirty-nine patients (26%) had associated cervical spine instability. Since cervical instability, together with narrowing of the spinal canal, can be associated with increased susceptibility to spinal cord injury following minor trauma, as well as myelopathy, MRI was recommended. Of the three patients who have had MRIs thus far, two had anterior and posterior narrowing of the spinal canal. CT imaging of both showed C1 abnormalities with lack of fusion of the anterior arch, lack of fusion of the posterior arch, changing the configuration of the ring, resulting in a narrow canal. Surgical intervention was recommended for these two patients.

Cervical spine x-rays including flexion-extension images are recommended in all patients with the 22q11.2 deletion, 3 years of age and older, in order to promptly identify and manage those with an increased risk for complications. Cervical spine abnormalities in these patients may be due to developmental genes in the deleted region whose role is important in signaling for segmentation and resegmentation of the cervical vertebrae.

Comparison of fatness patterning between obese and Prader-Willi syndrome subjects: Deletion versus UPD. Z. Talebizadeh, D. Bittel, N. Kibiryeve, M.G. Butler. Children's Mercy Hospitals and Clinics and University of Missouri-Kansas City School of Medicine, Kansas City, MO.

Prader-Willi syndrome (PWS), the most common cause of severe obesity in humans, is usually due to a deletion of chromosome 15q11-q13 or maternal disomy 15 (UPD). Obesity is due to energy imbalance but few studies have examined the regulation of fat metabolism or patterning in PWS subjects with deletion or UPD. We examined for differences in fatness patterning and correlation with lipid and endocrine data in three groups of subjects (obese controls, PWS-UPD and PWS-deletion). Fasting blood samples and cross-sectional MRI scans at the level of the umbilicus were obtained in 56 subjects: 21 PWS-deletion (9 males, 12 females; average age of 22y), 17 PWS-UPD (9 males, 8 females; average age of 21y) and 18 obese controls (8 males, 10 females; average age of 28y). Areas of subcutaneous fat (SFA), intra-abdominal visceral fat (VFA) and their ratio (SFA: VFA) were calculated. No significant differences were observed for SFA, VFA and their ratio among the three groups. Fasting plasma lipid, glucose and insulin levels were not significantly different in the three groups; however, triglyceride levels were lower in the PWS-deletion group compared with controls ($p=0.04$). Both SFA and VFA were positively correlated with insulin in the PWS-UPD ($r=0.99$; $p=0.10$ and $r=0.98$; $p=0.12$, respectively) and the PWS-deletion ($r=0.38$; $p=0.22$ and $r=0.37$; $p=0.24$, respectively) groups. In contrast, in the control group a negative trend and a flat association were seen, respectively, between SFA and insulin ($r=-0.23$; $p=0.40$) or VFA and insulin ($r=0.02$; $p=0.95$). VFA was significantly positively correlated with total cholesterol in the PWS-deletion group ($r=0.55$; $p=0.01$). This significant association was not observed in the UPD ($r=0.11$; $p=0.71$) or obese control ($r=0.17$; $p=0.52$) groups. In addition, growth hormone (GH) levels were significantly negatively correlated with VFA in PWS ($r=-0.56$; $p<0.01$) and in the obese control ($r=-0.72$; $p<0.05$) groups. GH deficiency is common in PWS and may contribute to abnormal fat patterning and reduced fat oxidation.

Development of original computer software "GenDis English version" useful in the diagnosis of congenital anomalies. *K. Naritomi¹, T. Kaname¹, Y. Chinen², T. Tohma²*. 1) Dept Medical Genetics, Univ Ryukyus Sch Medicine, Okinawa, Japan; 2) Dept Pediatr, Univ Ryukyus Sch Medicine, Okinawa, Japan.

At 51st Annual Meeting of ASHG, last year, we introduced our original computer software "GenDis" for diagnosis of congenital anomalies. Following an input of all clinical findings of the patient, the GenDis automatically selects candidate syndromes in order of increasing matched number of findings. Applying GenDis in out- and inpatient with congenital anomalies produced a good result, i.e. an appropriate diagnosis was suggested in 64% of each 393 patients tested. Since the GenDis is programmed using Japanese OS-driven Macintosh computer, this software could not use in English OS-driven Macintosh as well as Windows OS computers. Thus, we reprogrammed it for English version, both for Macintosh and Windows computers. The reference data included were quoted from our original database, UR-DBMS version 9, which has a data through April 1, 2002. These data have been updating everyday mainly through O-MIM in the web. The GenDis includes clinical findings, comments and reference data more than 7,000 diseases. It adopted two reference systems, one is referring from all findings without requisite findings and the other with requisite findings (maximum 5). The answer will be returned within about 10 seconds. Application of the GenDis will be a useful diagnostic tool in clinical genetics for quick diagnosis and further examinations to confirm the diagnosis.

Osteopathia Striata with Cranial Sclerosis: A brief clinical report on a Bedouin girl. *R.L. Alnaggar¹, S. Al-Awadi¹, M. Abu-Henedi¹, S. Gouda¹, H. Fathi², L. Bastaki¹.* 1) Obstetric, Kuwait Medical Genetic Center, Kuwait, Kuwait; 2) Pediatric Department Aljahra Hospital.

Objective: This report describes a case of Osteopathia striata with cranial sclerosis (OS-CS) in a Bedouin girl. Our case could be the first to be reported from Kuwait Genetic Center. **Methods:** Clinical examination, skeletal survey, echocardiography, ultrasonography and chromosomal analysis were carried out. **Results:** The patient had overgrowth of the craniofacial bones, developmental delay, hearing impairment, VSD and repeated attacks of seizures. **Radiographic findings** included marked ossification and sclerosis of the base of the skull and metaphyseal widening of the long bones with linear striations. **Conclusion:** Our case represents the typical form of the syndrome with dominant inheritance and could be added to the countable cases all over the world.

POLYSYNDACTYLY, COMPLEXE CARDIOPATHY AND HEPATIC DUCTAL PLATE ANOMALIES : AN AUTOSOMAL RECESSIVE SYNDROME. *B. GASSER, C. STOLL.* Medical Genetics, Hpital de Hautepierre, STRASBOURG, France.

A distinct syndrome was ascertained in a 3 year-old girl and her brother. The proband was the first child of first cousin parents. She was born after an uneventful pregnancy. At birth multiple congenital anomalies were noted : ptosis of the left eyelid, hypertelorism, anteverted nares, large fontanel, long philtrum, ungueal hypoplasia, polysyndactyly, simian crease, complex cardiopathy and hepatic cysts. During another pregnancy of the mother, fetal ultrasonographic examination showed an hypertrophy of the right ventricle and atria, a dextroposition of the aorta, a bilateral pyelic dilatation and a club foot. After termination of the pregnancy, necropsy showed a facial dysmorphia, a small penis, a polysyndactyly, a ventricular septum defect and a malformation of the ductal plate. Bonneau et al. (1983) described a family in which 3 sibs had a complex cardiac malformation, hexadactyly of the first toe, and syndactyly of the third and fourth fingers. Rejab (1997) described two sibs with similar features in an Omani family. The sibs described in this report had anomalies of the ductal plate which were not reported in the two other families. These new findings are in favour of autosomal inheritance of this condition which is amenable to antenatal diagnosis.

An unusual type of XY sex reversal associated with the presence of female gonads. *J.C. Ferreira¹, M. Nicolau², B. Marques², P. Borralho¹, P. Rendeiro³, C. Nunes¹, F. Torres³, R. Lemos³, J. Gonçalves².* 1) Obstetrics and Gynecology, Garcia de Orta Hospital, Almada, Portugal; 2) Centro de Genética Humana, INS Dr. Ricardo Jorge, Lisboa, Portugal; 3) Centro de Genética Clínica, Porto, Portugal.

Molecular studies performed in patients with gonadal dysgenesis or with sex reversal have been providing some hints on the complex molecular mechanisms of human sex determination pathway. A few genes have been identified (ex. SRY, SOX9, SF1, WT1, WNT4, DMRT1,2) and mutations in these genes in XY individuals usually cause complete gonadal dysgenesis or partial gonadal dysgenesis associated with completely female phenotype or with ambiguous phenotype, respectively.

We report a fetus with a unique combination of anomalies, which also had an unusual XY sex reversal.

Case report

A 22 week fetus was found with severe oligohydramnios, polycystic kidneys and hypoplastic left heart. A karyotype from cultured fibroblast cells showed a 46,XY karyotype. Postnatal evaluation confirmed the prenatal findings. Facial dysmorphism and normal female internal and external genitalia was found. Gonad histology, revealed two ovaries with primary oocytes. No signs of dysgenesis and testis tissue were found. The skeletal X-ray survey was normal. Tissue interphase FISH demonstrated the presence of Y chromosome material in all ovarian cells analyzed. Molecular studies of DNA extracted from fetal ovaries and liver evidenced the presence of the SRY gene. DNA sequencing of the SRY HMG domain was normal.

This unusual XY sex reversal may be caused by a mutation in a unknown gene involved in the morphogenesis of several organs and in male gonad development. Other explanation could be a continuous gene deletion syndrome that would include a new sex determination gene. It seems that lack of function of an unknown gene, involved in primary sex determination, apparently suppressed the SRY testis determining action and allowed the development of female gonads.

Abnormal trajectory of the internal carotid within the middle ear in a patient with Smith-Magenis syndrome. V.

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The Smith-Magenis syndrome results from an interstitial deletion within the 17p11.2 chromosomal region. Clinical features include mental retardation, particular facial features (flat mid-face, prominent jaw), behavior problems with hyperactivity and self injuries, severe sleep disturbance, and heart defect. Hearing impairment, resulting from conductive and/or sensory neural hearing loss, is frequently observed. We report the case of a patient who was born at 36 weeks after an uneventful pregnancy. Neonatal course was marked by respiratory distress secondary to laryngomalacia, and auricular septal defect. Dysmorphic facial features were obvious with mid-face hypoplasia and macroglossia. High resolution chromosomal examination showed a 17p11 deletion. At 18 months of age, she had developmental delay. Audiometric testing was performed showing mixed hearing loss. Otoscopy showed bilateral retro-tympanic mass and CT scan revealed an aberrant course of the internal carotid within the middle ear. Only about fifty cases of this vascular mal-positioning have been reported so far. It is the first reported case associated with the Smith-Magenis syndrome. This report suggests that careful otorhinolaryngological examination should be recommended in Smith-Magenis patients to prevent the risk of major haemorrhage and potential neurological complications.

LETHAL SYNDROME WITH ABLEPHARON: A VARIANT OF THE ABLEPHARON MACROSTOMIA SYNDROME OR AN INTERMEDIATE FORM BETWEEN THIS ONE AND THE FRASER SYNDROME?

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The eyelids are structures developed from two small folds of ectoderm with mesenchymal core in the sixth week of embryonic development. The absence of eyelids, also designated as ablepharon, is a rare abnormality described in few syndromes. In this report a male stillbirth presenting multiple anomalies including absence of eyelids is described. This male stillbirth with 22 weeks of gestation was the product of the first gestation of young and nonconsanguineous parents. There was no significant family history. Pregnancy was complicated by severe gestational hypertension. Ultrasound examination had shown oligohydramnios and fetal hydrops. Postmortem examination showed alopecia, redundant and ichthyosiform skin, specially in abdominal region and distal limbs, absent eyelids, telecanthus, hypoplastic alae nasi, flat malar region and philtrum, macrostomia, very low insertion of the umbilical cord, hypoplastic scrotum, abducted hips, skin syndactyly of fingers and toes (2nd, 3rd, 4th /5th) and contracture of the left elbow. Osteopenia and increased metaphyseal density in femur was observed in skeletal X-ray. The necropsy showed laryngeal and epiglottal agenesis, tracheomalacia, esophageal stenosis, lung hyperplasia, malrotation of small intestine, bilateral renal agenesis and hypoplastic bladder. Microscopic examination showed disorganization of the ossification centers of the long bones and thickening of the stratum corneum of the epidermis. The placenta was senescent and had a microcorangyoma. The karyotype, performed from peripheral blood lymphocytes, was normal (46,XY). The phenotype of this case is not compatible with the syndromes associated with ablepharon. However, the dysmorphologic analysis suggests that it is a lethal variant of the Ablepharon Macrostomia syndrome or an intermediate form between this one and the Fraser syndrome. (Grants : FAPESP, # 98/16006-6).

Phenotypic overlap with central polydactyly in Bardet-Biedl and McKusick-Kaufman syndromes. *M. Gerard*¹, *C. Barrey*², *C. Deffert*³, *F. Niel*³, *E. Girodon*³, *C. Romana*⁴, *S. Amselem*³, *M. Le Merrer*⁵. 1) Medical genetics, Neonatology, CHIC, Creteil, France; 2) Pediatrics, Hopital Sainte Camille, Bry sur Marne, France; 3) Molecular genetics, Hopital Henri Mondor, Creteil, France; 4) Pediatric surgery, Hopital Trousseau, Paris, France; 5) Medical genetics department, Hopital Necker-Enfants Malades, Paris, France.

The fifth child of a consanguineous marriage has mild mental retardation, situs inversus, feet postaxial polydactyly, and central polydactyly by Y duplication of the third metacarpal. The eldest brother had the same malformative pattern, acquired blindness, and died at 22 years of renal failure. The other siblings are healthy. Clinical examination shows hypogenitalism with micropenis and atrophic testes, obesity (plus 4 SD), normal stature and OFC (M). Mild facial dysmorphism is noted, with downturned corners of mouth, short nose with anteverted nostrils and midface hypoplasia. Dyschromatopsia is present, and ERG is extinguished. The diagnosis of Bardet-Biedl syndrome (BBS) was first raised, given the association of mental retardation, polydactyly, hypogenitalism, obesity, with secondary apparition of renal failure and blindness. However, situs inversus has been documented in only 3 cases of BBS (Lorda-Sanchez et al., 2000). Central polydactyly is not a feature of BBS, but is frequent in the McKusick-Kaufman syndrome (MKKS), which associates hydrometrocolpos in females, cardiopathy and polydactyly. Intermediate forms between BBS and MKKS had been described (David et al., 1999; Slavotinek and Biesecker, 2000). At least, seven locus (*BBS1-7*) are involved in BBS, while one (*BBS6/MKKS*, 20p12) is involved in MKKS (Slavotinek et al., 2000). Triallelic inheritance for BBS has been recently proved, patients being homozygotes or compound heterozygotes at one BBS locus, and heterozygotes at another BBS locus (Katsanis et al., 2001). In the present case, microsatellite analysis at the *BBS6/MKKS* locus showed heterozygosity. Investigations of the other known loci are in process. This familial observation, with situs inversus and central polydactyly, will further document the molecular bases of the Bardet-Biedl/McKusick-Kaufman syndrome.

Gonadal Mosaicism in Pallister-Hall Syndrome. *D. Ng, J.J. Johnston, J.T. Turner, L.G. Biesecker.* GDRB/NHGRI, National Institutes of Health, Bethesda, MD.

Pallister-Hall syndrome [PHS, MIM #146510] is characterized by central polydactyly, postaxial polydactyly, hypothalamic hamartoma [HH], asymptomatic bifid epiglottis, imperforate anus, renal abnormalities, and pulmonary segmentation anomalies. It is inherited in an autosomal dominant pattern. Here we describe a family with two affected children manifesting HH, bifid epiglottis, brachydactyly, and imperforate anus. Atypical features include severe mental retardation, behavioral problems, and difficult to control generalized seizures. Both parents are healthy, with normal intelligence, and have no identifiable malformations on physical, laryngoscopic, and cranial MRI exam. The atypical presentation of these children coupled with the absence of parental manifestations suggest an autosomal recessive mode of inheritance or gonadal mosaicism. Sequencing of the *GLI3* gene revealed a 2 nucleotide deletion in exon 15 [c.3385_3386delTT] resulting in a frameshift and premature stop codon at aa 1129 [p.F1129X] in the children while both parents have wild type alleles. Genotyping with *GLI3* intragenic STRPs revealed that both children inherited the abnormal allele from their mother thus supporting gonadal mosaicism as the underlying mechanism of inheritance and paternity was confirmed. This is the first reported case of gonadal mosaicism in Pallister-Hall syndrome. The severe CNS manifestations of these children are reminiscent of individuals with non-syndromic or isolated HH who oftentimes develop progressive mental retardation with behavioral problems and have seizures that are refractory to medical management. We conclude that the phenotype of PHS can include severe CNS manifestations of intractable seizures and that recurrence risks for PHS must include a proviso for gonadal mosaicism, though the risk cannot be calculated from a single case report.

A Japanese boy with Noonan syndrome with abnormally high urinary VMA and HVA levels. *T. Kondoh¹, E. Ishii², Y. Aoki³, T. Shimizu¹, T. Matsumoto⁴, Y. Matsubara³, H. Moriuchi¹.* 1) Dept Pediatrics, Nagasaki Univ Sch Medicine, Nagasaki, Japan; 2) Dept Pediatrics, Saga Med Sch, Saga, Japan; 3) Dept Med Genet, Tohoku Univ Sch Medicine, Sendai, Japan; 4) Dept Nursing, Nagasaki Univ Sch Medicine, Nagasaki, Japan.

Noonan syndrome (NS) is a malformation disorder characterized by dysmorphic facial features, webbed neck, cubitus valgus, short stature and heart disease. Recently PTPN11 was identified as the causative gene, encoding the nonreceptor protein tyrosine phosphatase SHP-2. NS has also been associated with malignant diseases including a number of cases of lymphoblastic leukemia, and a few cases of neuroblastoma. We here report a Japanese boy with NS with high urinary catecholamine levels. He has a novel de novo missense mutation (Ser502Thr) in PTPN11 gene, never found in 77 normal controls. His urinary VMA and HVA at the age of six months in nation-wide mass screening test were 27.7 and 29.1 ug/mgCre, respectively. MRI showed an 8-mm-diameter mass in his left adrenal gland, which was compatible with neuroblastoma. His tumor spontaneously disappeared radiographically during the next 6 months. Despite regression of the mass, his VMA and HVA levels have been increasing gradually. Our case might indicate that SHP-2 is involved in catecholamine metabolism or proliferation of catecholamine-producing cells, and that certain mutations of PTPN11 gene may result in neuroblastoma formation or overproduction of catecholamines.

Unusual cardiac presentation of an infant with tuberous sclerosis. *J. Lindsey, E. Pivnick, L. Winkeler, J. Ring, D. Nelson.* Department of Pediatrics, University of Tennessee, Memphis, TN.

Tuberous sclerosis complex (TSC) is a systemic disorder characterized by widespread hamartomas. Cardiac rhabdomyomas (CR) are known to be associated with TSC. These tumors tend to regress in the first year of life. The in-utero growth pattern and self-limiting proliferation of CR is not well understood. We report an infant with TSC who presented with an unusual form of tachycardia, chaotic atrial rhythm (CAR), and widespread intra-myocardial lesions. The male infant was born at 37 weeks gestation to a 26 year old mother. The mother's HIV status was discovered at 33 weeks. She received zidovudine (ZVD) for the remainder of the pregnancy. The neonatal course was complicated by pulmonary hypertension requiring mechanical ventilation. The infant was discharged home at 16 days to complete 6 weeks of ZDV for HIV prophylaxis. At age 7 weeks the infant presented with bronchiolitis. He had tachycardia at 210/min and a few hypo-pigmented skin maculae. Soon after admission he developed CAR. Holter monitoring showed >100 episodes of described rhythm. Infant was begun on propranolol therapy. Echocardiogram revealed multiple echodense lesions consistent with very small intra-myocardial CR of the left ventricle. Because of the suspicion of TSC, an MRI of the head was obtained and revealed many subcortical tubers. Fundoscopy illustrated an astrocytoma of the right retina. At 6 months of age the CAR has resolved and the myocardial lesions have disappeared. It has been demonstrated that ZDV preferentially incorporates in telomeric sequences of immortalized cell cultures derived from certain tumors and makes those cells more susceptible to apoptosis. It has been proposed that CR is due to delay or failure of apoptosis that occurs as part of the normal remodeling process in the heart. It has been shown, however, that the regression of CR is due to an increased rate of cell death. It is intriguing to speculate that the antiretroviral therapy this infant received may have accelerated this process. Conversely, the intra-myocardial changes may be just one end of the spectrum of CR in TSC. This is the first report of CAR associated with TSC.

A new case of intractable infant diarrhoea associated with phenotypic abnormalities and immunologic disorders.

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The syndrome of Intractable Diarrhoea of Infancy (IDI) is an entity that includes Microvillous inclusion disease, Epithelial dysplasia, Syndromatic diarrhoea and Autoimmune enteropathy. We report the case of a one-year-old girl highly suspected of syndromatic diarrhoea (J Pediatr 125:36-42,1994) in front of the association of intrauterine growth retardation, IDI, facial dysmorphism, hair abnormalities and immunological disorders. The girl, from Kurd origins and issued from a consanguineous marriage, was born on 35 weeks of gestation, with a birth weight of 1050 g (<<-5c). Her first weeks of life were characterized by the onset of a severe, watery, bloodless diarrhoea, persisting despite bowel rest and requiring total parenteral nutrition. Clinically, she presented the classical phenotype with prominent forehead and cheeks, hypertelorism and a broad nose associated with woolly, poorly pigmented and undressable hair. However, microscopic analysis of the hair observed an aspect of pili torti and not the trichorrhexis nodosum usually found. Repeated intestinal biopsies showed a partial villous atrophy but without the typical features seen in the other IDI. Immunologic analyses revealed a complete lack of sub-class of immunoglobulins G and a weak antibody response to antigens. Standard karyotype, skeletal X-Rays and metabolic screening were then normal. Finally, among the other features non usually associated with the syndromatic diarrhoea, the child presented a spina bifida occulta, bilateral ovary herniae, transitory hypothyroidism- without any manifestation of autoimmunity- and a persistent arterial canal requiring surgery. The girl is now stabilised, starting back growing with home parenteral nutrition supporting minor oral feeding. Therefore, after excluding the other types of IDI but also primary or acquired immunodeficiencies, we concluded on a new case of syndromatic diarrhoea. It is to be noted that, on nine cases reported, here is the third case occurring in a consanguineous family so that genetic counselling should be quite careful.

Bilateral asymmetry in a Chinese sample with nonsyndromic cleft lip with or without cleft palate. K.

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Increased bilateral asymmetry may be seen in individuals with diverse congenital anomalies. We employed a case - control study design in order to determine if Chinese individuals with nonsyndromic cleft lip with or without cleft palate (CL/P) displayed more bilateral asymmetry than their unaffected relatives. Left and right mid-finger length, palm length, palpebral fissure width, and ear length were collected from 313 CL/P probands and 201 unaffected relatives in Shanghai, China. Asymmetry scores between right and left sides were defined for each of the four anthropometric measures by calculating the quotient of the right measurement divided by the left. Asymmetry quotients were compared between probands and their unaffected relatives using standard t tests, paired t tests and multivariate repeated measures analysis (ANOVA).

For all four measures, the probands' asymmetry scores did not differ significantly from the scores of unaffected relatives utilizing standard t tests. Paired t tests on a subset of 187 pairs of probands matched to their unaffected relatives also showed no significant differences for any of the measures. Subdivision of the sample according to gender, cleft type, and laterality did not change the results. However, when gender-matched proband/relative pairs (n = 74 male-male or female-female pairs) were analyzed by ANOVA, there were significant differences in the asymmetry scores for palpebral fissure width (p = 0.007), that depended both on cleft type (p = 0.004; CL alone vs CL/P) and laterality (p = 0.0001; unilateral vs bilateral). These differences were not seen in the non-gender matched sample (n = 113 male-female and female-male pairs). Supported by NIH grant# DE-09886.

A study of congenital cataract in 5000 newborns in Mashhad Hospitals, North East of Iran. *G.Ali. Mamoori¹, K. Ghodsi², A. Derakhshan³, F. Behmanesh¹, R. Akbarzadah², S. Mosavian¹, P. Sasan Nejad¹.* 1) Pediatric, Mashhad University of Medical , Mashhad, Khorasan, Iran; 2) Medical genetics, Mashhad University of Medical , Mashhad, Khorasan, Iran; 3) Ophthalmology, Mashhad University of Medical , Mashhad, Khorasan, Iran.

A study of congenital cataract in 5000 newborns in Mashhad Hospitals, North East of Iran. Gholam Ali Mamoori¹ Kazem Ghodsi², Akbar Derakhshan³, Fatemah Behmanesh¹, Reza Akbarzadeh², Saied Javad Mosavi Baigy¹ and Payam Sasan Nejad¹. Department of Pediatric¹, Department of Medical Genetics², and Department of Ophthalmology³, Ghaem and Emam Reza Hospitals, Faculty of Medicine, Mashhad University of Medical Sciences Mashhad Iran. Here we presented a study of congenital cataract in 5000 newborns in three major Mashhad Hospitals, 2000 to 2001 year. Twelve infants have been diagnosed with congenital cataract. Clinical studies showed that 45% of patients affected with bilateral eyes, three with right eyes and two patients had left eyes affected. Five infants had other abnormalities including cleft palate, cleft lips, microcephaly, polydactyly, bilateral cryptorchidism, VSD, hydrocephaly, hepatosplenomegaly, low seat ear, high arch palate, microstomy, calcification in around brain ventricular in CT scan. Patients study by gender showed that 55.5% were female and 44.5% male. Consanguinity study showed one patient with first cousin marriage parents and clinical findings in this patient were one eye affected, VSD, cleft palate, 1700 grams weight, lower limb hyperplasic trisomy 13. Age of pregnancy was in 55% were less than 37 weeks and 66% of infants had less than 2500 grams weight. To study chromosomal abnormalities peripheral blood samples from patients were taken and cells were harvested, GTG banding was carried out. Cytogenetic results showed two patients with trisomy 13 and the rest did not reveal any abnormal chromosome. DNA samples from patients and families were stored for future molecular study. This project is one of our current projects.

LIMB REDUCTION DEFICIENCIES IN THE SECOND GENERATION AND DEAFNESS IN THE THIRD GENERATION OF INTRAUTERINE EXPOSED FETUSES TO DIETHYLSTILBESTROL. C. STOLL, Y. ALEMBIK, B. DOTT. Medical Genetics, Hpital de Hautepierre, STRASBOURG, France.

Diethylstilbestrol (DES) was widely prescribed to pregnant women between 1938 and 1975 for treatment of imminent and habitual abortions. It is well known since the early 1970s that maternal treatment with diethylstilbestrol during pregnancy can produce vaginal adenocarcinoma and other abnormalities of the vagina in her daughters when they reach adolescence or adulthood. Concerning malformations in newborn whose mothers were treated with DES, clitoromegaly and malformations of the uterus were reported in female and genital lesions as well as pathological changes in spermatozoa in male. However the frequencies of major congenital anomalies were not greater than expected. We report 3 cases (2 males and 1 female) with limb reduction deficiencies (LRD) in the second generation of children whose mothers were treated with DES during pregnancy, and two children (1 male, 1 female) with deafness in the third generation after intrauterine exposure to DES. The LRD were not associated with other congenital anomalies and were of the terminal transverse type. LRD were not familial. The malformed children were born between 1965 and 1973. The two children with deafness, had sensorineural congenital deafness. Deafness was sporadic nonsyndromic. No genetic causes responsible for the hearing impairment were detected, i.e. mutation of CX26 or GJB2. These children were born in 1989 and 1994, respectively. They were not exposed to DES in utero. However their mothers, born in 1965 and 1963, respectively were exposed to DES in utero. This is the second example of transgenerational effect of DES in human beings, the first one being 4 children born with hypospadias, the sons of women exposed in utero to DES (Lancet, 2002;359:1102-7). Genetic or epigenetic changes in germ or (less likely) somatic cells of the exposed women are suggested as the biological explanation of this transgenerational effect of DES. Although DES is no longer used weaker estrogens are present in oral contraceptives, phyto-estrogens in the diet, and certain solvents or pesticides.

Anophthalmia/Microphthalmia(A/M) Registry: Results of first 2 years of gene screening. Is A/M biallelic? A.

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The A/M registry at Albert Einstein Medical Center (AEMC) was established in 1994 and has been offering gene screening to registry participants since 1999. Testing involves mutational screening of 17 eye development genes with 8 participating labs. The DNA is collected from the affected individuals and family members with buccal brushes and/or blood which is sent in the mail from the central clearing house at AEMC to each lab. Samples are sent to all labs with minimal pre-selection. Results from 5 of the participating labs are as follows: No mutations have been noted in the PITX2, PITX3, PAX6 genes and ATHO7. A few silent polymorphisms in VAX1 have been identified. Two heterozygotes with a de novo Q147X mutation in the RX gene have been documented. In the SIX6 gene one heterozygous intronic change, IVS1-66t/c, has been found. The finding of heterozygous mutations in affected individuals is unexpected as these are known to be autosomal recessive genes. This raises the question as to whether a mutation in another gene is present in each of these individuals and therefore the possibility of biallelic inheritance.

Congenital absence, abnormal shape and reduced size tooth as manifestation of same genetic defect. *D. Saavedra*¹, *E. Barberia*¹, *M. Orera*². 1) Odontopediatrics, UCM, Madrid, Spain; 2) Genetics, H. Gregorio Maranon.

INTRODUCTION. Tooth development is a complex process including several stages through which the primordial cell passes: initiation, proliferation, histodifferentiation, morphodifferentiation, apposition, calcification and eruption. These abnormalities may result from genetic or environmental factors or a combination of both, producing excessive or deficient development at those stages. Until now it's not clear the extent to which heredity influences the final size, form and number of teeth or the number of loci contributing to various dental normal and abnormal traits.

MATERIAL AND METHODS. A 11 years old male child was studied because his dental anomalies. He presented oligodontia since the primary dentition with lack of both superior lateral incisors and canines. These teeth were also missing in the permanent dentition. He also presented a delayed eruption of permanent teeth but the main feature was the abnormal shape of the two present superior incisors and the four lower incisors which showed a conic shape since the primary dentition. No facial or other malformations were present nor skin, hair or nails abnormalities. When the parents were examined a significant small size in all mother's teeth was noted. The familial pedigree showed an autosomal dominant transmission of this trait in the mother's side as well as a history of oligodontia, also dominantly inherited.

DISCUSSION There are several pedigrees showing an autosomal dominant inheritance of hypodontia as an isolated trait affecting third molars or lateral incisors of permanent dentition, but it is so rarely involving canines at the primary dentition as our patient had. There are also some pedigrees that related small size with hypodontia suggesting a dominant inheritance with incomplete penetrance and variable expression of the same gene defect, but there are no many instances where also an abnormal tooth shape were present at the same family. The present pedigree confirms these three abnormalities, as produced by the same autosomal dominant mutant gene with reduced penetrance and clinical variable expression from small dental size to conical shape or missing tooth.

Urorectal septum malformation sequence: a case suggesting a separate entity from caudal regression sequence and a developmental field defect etiology. *M.C. Wilson¹, R. Wilcox², G. Murdoch², M. Grompe¹*. 1) Molecular & Med Genetics/L103, Oregon Health & Sci Univ, Portland, OR; 2) Dept of Pathology/L113, Oregon Health & Sci Univ, Portland, OR.

The urorectal septum malformation sequence is a term originally proposed in 1987 by Escobar et. al. for a category of anomalies that include absence of the perineal and anal openings, ambiguous genitalia, and associated anomalies. An absence of mesodermal cell migration into the caudal region of the embryo has been proposed as the basic cause of the malformation. We present a case where the lack of perineal and anal openings and ambiguous genitalia are clearly seen, yet there is normal development of the sacrum and a lack of other associated anomalies. Despite a 46 X,X karyotype by amniocentesis, the genitalia consisted of a primitive small phallic structure without urethral structures, absence of labial or scrotal structures, and no vagina. Further we can see the persistence of the plane of tissue containing the bladder wall, uterus, and sigmoid colon where there is no development of the urorectal septum, apparently leading to lack of development of the perineal structures. This case is more consistent with the original proposal by Escobar of an abnormality in the induction of the formation of perineal structures and genitalia secondary to the lack of development of the urorectal septum. Further it suggests a separate entity from the caudal regression sequence with possibly separate etiological factors.

Inherited capillary malformation is associated with a high flow vascular malformation. *L. Boon*^{1,2}, *J.B. Mulliken*³, *A.Ch. Bataille*², *G. Vittu*⁴, *R. Vanwijck*², *M. Vikkula*¹. 1) Center for Vascular Anomalies, Université catholique de Louvain, Brussels, Belgium; 2) Laboratory of Human Molecular Genetics, Christian de Duve Institute and Université catholique de Louvain, Brussels, Belgium; 3) Division of Plastic Surgery, Center for Vascular Anomalies, Children's Hospital, Boston, MA, USA; 4) Division of Neonatology, Hôpital Pédiatrique Saint-Antoine, Université catholique de Lille, France.

Capillary malformation (CM, portwine stain) is a common cutaneous vascular anomaly occurring in 0,3% of neonates. Except for rare reports of familial nuchal CM (stork bite), CM is considered sporadic. Clinical diagnosis is usually straightforward, however, CM can masquerade a high-flow vascular lesion, i.e., stage one AVM (arterio-venous malformation) or AVF (arterio-venous fistula). We identified several families with inherited CM. We clinically examined each family member, noted all vascular anomalies and collected informed consents and blood samples. Inter- and intrafamilial variation was observed, from nuchal CM persisting throughout life and extending below the hairline, to solitary or multiple CM disseminated all over the body. Seven of our families with inherited CM had at least one member affected with a high-flow vascular malformation. Three of them had either AVM (n=2) or AVF (n=1). Four families had two members with AVM (n=8) including Parkes Weber syndrome (n=3), AVM with multiple CM (n= 3), or intraosseus AVM with cutaneous blush (n=1) or extensive CM (n=1). These data confirmed autosomal inheritance of CM. In addition, the high incidence of high-flow vascular malformations observed within families with inherited CM suggests genetic predisposition for AVM and/or AVF in these families. (vikkula@bchm.ucl.ac.be) (<http://www.icp.ucl.ac.be/vikkula>).

Mosaicism for deletion of 15q11.2q13 in a patient with atypical Angelman Syndrome phenotype: A second case.
L.K. Berkheim, S.O. Lewin, B. Issa, E. Drozd-Borydiuk, A. Meloni-Ehrig, R. Mao, A.R. Brothman, J.C. Carey. Division of Medical Genetics, University of Utah, Salt Lake City, UT.

We report a young girl with mosaicism for a deletion 15q11.2q13 involving the PWS/AS critical region. Our patient has global developmental delay with language more severely impaired than motor skills, normal growth with head circumference at the 95th centile, unusual gait and sleep disturbance. Her facial features include a long philtrum with a thin upper lip, prominent forehead and wide mouth. A seizure disorder was recognized at 3 years of age with an EEG pattern consistent with Angelman syndrome (AS). Chromosome analysis on peripheral blood lymphocytes indicated mosaicism with a visible deletion at 15q11.2q13 at the 550 band level in 35% of cells. FISH studies showed mosaicism for a deletion in the AS critical region (SNRPN/D15S10) in 50% of scored cells. PCR based methylation studies indicated a normal biparental methylation pattern, although the sample showed slightly reduced amplification of the maternal signal, supporting that the deletion is maternally derived. Tekin et al. (*Am J Med Genet* 95:145-149, 2000) reported a similar case with normal methylation pattern and mosaic deletion of the AS critical region detected by FISH. Our patient closely resembles the reported case in facial features and developmental profile. Both cases appear to have a milder presentation compared to typical AS patients. In summary, methylation studies are commonly used as an initial screening for AS because most cases show only paternal methylation regardless of the molecular explanation for the condition. However, these two cases demonstrate that methylation studies alone are not sufficient to rule out AS. High resolution chromosome analysis with FISH should also be performed in patients with clinical features of AS despite a normal methylation pattern.

Role of a COL5A1 polymorphic variant(G530S) in the classical Ehlers-Danlos phenotype. *A.L. Mitchell, P.H. Byers.* Medical Genetics, University of Washington, Seattle, WA.

Classical Ehlers-Danlos syndrome (EDS type I/II) is characterized by soft, hyperextensible skin, hypermobile joints, and abnormal scar formation. Most affected individuals have mutations in the type V collagen genes (COL5A1 and COL5A2). In the COL5A1 gene these mutations result in substitutions for glycines in the triple helical domain, exon skipping, or haploinsufficiency mutations, while in the COL5A2 gene, most mutations alter triple helical glycine residues. One mutation in the COL5A1 gene leads to skipping of two exons (exons 5 and 6) in the region that encodes the amino-terminal propeptide of the pro α 1(V) chain. A second amino-terminal sequence variation (substitution of serine for the glycine at position 530, G530S) has recently been proposed to influence the expression of a mutation on the other allele and, in the homozygous state, to produce the classical EDS phenotype itself. To better understand the role of this variant sequence, we determined its frequency in individuals with inherited connective tissue disorders and in other groups without these disorders. Among 72 individuals with classical EDS there were 6 530S alleles (4.2%); 8/188 (4.2%) alleles in people with EDS type IV, and 9/164 (5.5%) alleles in people with osteogenesis imperfecta. Study of DNA samples from individuals without connective tissue disorders identified 16/300 (5.3%) of the COL5A1 alleles as the 530S variant. One of the anonymous DNA samples was homozygous for the variant. The incidence of homozygosity of 1/398 individuals is consistent with the allele frequency in the population studied. In an individual with classical EDS and a COL5A1 haploinsufficiency mutation, the 530S variant was on the unstable allele. A COL5A1 or COL5A2 mutation has not been identified in the other 5 classical EDS patients, but the presence of the 530S variant did not appear to modify the phenotype. These studies suggest that, contrary to published data, the COL5A1 530S allele has little role in modifying the classical EDS phenotype.

Redefining the MFS2 Locus at 3p24.2-25 as a Second Locus for Familial Thoracic Aortic Aneurysms and Dissections (TAAD2). *S.N. Hasham¹, A. Muilenberg², R. He¹, V. Tran¹, M. Willing², S. Shete³, D.M. Milewicz¹.* 1) Internal Medicine, Univ TX Med Sch Houston, Houston, TX; 2) Dept of Pediatrics, Univ of Iowa, Iowa; 3) Dept of Epidemiology, MD Anderson Cancer Centre, Houston, TX.

Marfan syndrome (MFS) is an autosomal dominant disorder that affects the ocular, skeletal and cardiovascular systems. MFS results from mutations in *FBN1* on 15q21. A second locus for a MFS-like syndrome was mapped to 3p24-25 using a large French family (MFS2 locus), raising the possibility of genetic heterogeneity for MFS. The data concerning the clinical characterization and molecular studies of the French family was controversial, raising doubts about the validity of the mapping of the locus and questions about the associated clinical phenotype. We studied a family of 4 generations with 81 members with autosomal dominant inheritance of thoracic aortic aneurysms and dissections (TAAD), in which the phenotype was not linked to any of the identified loci for TAAD. None of the affected members meet the diagnostic criteria for MFS. Affected and unaffected status was based solely on the cardiovascular features, i.e., aortic aneurysm or dissection or aortic root dilatation. A genome wide screen, using 400 highly polymorphic markers spaced approximately 10 cM throughout the human autosomes, was completed and LOD scores were obtained using the MLink program under the assumption of an autosomal dominant model with age related penetrance. Loci demonstrating a LOD score >0.6 were investigated further using markers spaced 1-3 cM apart. TAAD in this family was linked to a 30 cM region at 3p24-25, directly overlapping the MFS2 locus. Linkage analysis demonstrated eleven markers in the region with a positive LOD score above 3.0 and a maximum multipoint LOD score of 3.68 was obtained with marker D3S2336. The data confirms the existence of a locus on 3p for familial TAAD with reduced penetrance of the cardiovascular manifestations. In addition, the clinical phenotype associated with the locus in our family is not MFS, but rather TAAD.

Characterization of DPH Syndrome: a new autosomal dominant constellation of dermatoglyph hypoplasia and palpebral hypertrichosis. *J.P. Doucet¹, A.K. Lirette¹, S.M. Robichaux¹, A. Duke¹, R. Self II¹, Y. Lacassie^{2,3}.* 1) Biological Sciences, Nicholls State University, Thibodaux, LA; 2) Pediatrics, LSU Health Sciences Center, New Orleans, LA; 3) Children's Hospital, New Orleans, LA.

We report a large family expressing a unique constellation of dermal abnormalities. The cardinal features of the syndrome include dermal ridges hypoplasia of both the palmar and plantar surfaces, distichiasis and hypertrichotic growth across the eyelids as the brows thicken and descend toward the lateral canthi, raised and pyramidal fingerpads and toepads, underkeratinized and sweaty palmar and plantar surfaces, dorsal hyperpigmentation of the phalangeal surfaces, excessive tearing of the eyes, and coarse body hair. The syndrome affects at least four generations of an extended southern Louisiana family of Acadian descent. This unique unreported constellation of abnormalities, inherited in an autosomal dominant pattern, suggests the presence of a new syndrome.

KID syndrome (Keratosis, Ichthyosis and Deafness). *P. Lenane*¹, *S. Cammisuli*¹, *B. Krafchik*¹, *A. Pai*², *D. Chitayat*².
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KID syndrome is a rare, congenital ectodermal disorder, consisting of the triad of a vascularising keratitis, ichthyosis and deafness. The condition is most probably autosomal dominant with full penetrance and most cases were the result of a new dominant mutation. We report an additional case of this rare condition detected prenatally with Dandy-Walker malformation. The patient was born to a non-consanguineous couple of Portuguese descent and the pregnancy was complicated with fetal ultrasound finding of Dandy-Walker malformation. Delivery was spontaneous and vaginal at term. After delivery the female newborn was found to have dystrophy of her finger and toe nails, erythematous scaly papules on her palms and soles and papillomatous hyperkeratosis covering her scalp, trunk and limbs. She had multiple milia extending from the bridge of her nose across both cheeks, onto her pinnae and filling both external ear canals. Peribuccal grooves, sparse hair growth on the scalp and alopecia of the eyebrows and eyelashes were also noted. The diagnosis of a Dandy Walker malformation was confirmed but no other fetal abnormalities were detected. The cutaneous findings evolved over 3 months with resolution of the milia and the generalized papillomatous hyperkeratosis. Normal nail growth has been noted on 2 fingernails but she has developed spiny processes on the palms of the hands and soles of her feet. Thickened areas of hyperkeratosis have developed at the angles of her mouth and across her eyebrows. She has recurrent episodes of conjunctivitis and bacterial and candidal skin infections. Auditory evoked responses have confirmed the presence of profound sensorineural deafness.

Misoprostol embryopathy: clinical evaluations of 13 patients with congenital hydrocephaly. *C.H. Gonzalez^{1,2}, M.J. Marques-Dias², C.A. Kim², J.A. Paz², S.M.M. Sugayama², M. Valente².* 1) Dept. Biology. Instituto de Biocincias - USP -Brazil; 2) Dept.of Pediatrics - Instituto da Criana. University of So Paulo - So Paulo -Brazil.

Misoprostol, a synthetic analog of prostaglandin E1 has been misused as abortifacient in Brazil, where abortion is not a legal procedure. The drug is used, in the vast majority of the cases, in not medically controlled conditions, with a great percentage of failure. We have been studying and have already published(a,b) the clinical findings of children with birth defects exposed in utero to misoprostol. In a consecutive series of 67 patients evaluated between June, 1992 and April, 2002, congenital hydrocephaly was diagnosed in 13 cases (19,4%). Among them, 11 also presented equinovarus feet. Nine had cranial nerve deficiencies, most involving the fifth to seventh nerves (Mbius anomaly); 5 had arthrogriposis. One patient presented syndactylies of the fingers and another of the toes. Ten of these children's mothers took 400-800 mcg of misoprostol (Cytotec) orally or vaginally at 4-12 weeks of pregnancy. One mother took 8000 mcg orally and 8000 vaginally. In 9 patients the imaging study showed a non-communicating hydrocephaly with signs of aqueduct stenosis. Other cerebral abnormalities were also seen . Cranial nerve deficiencies (Mbius anomaly), terminal transverse limb defects, constriction bands, arthrogriposis, equinovarus, amyoplasia and hydrocephaly are characteristics of misoprostol embryopathy, a complex phenotype not yet fully described. These deformities can be attributed to a mechanism of vascular disruption provoked by the uterine contractions induced by misoprostol. A question to be answered is: can a vascular disruption phenomenon, explain all these findings? Another question, an estimate of the potential teratogenicity of misoprostol is still to be determined. aGonzalez et al. *Am J Med Genet*, 1993; 47: 59-64, bGonzalez et al. *Lancet* 1998; 351: 1624-1627.

Dysmorphological diagnosis of fetal alcohol syndrome (FAS) in two ethnic groups: African-Americans and Caucasians. *R.D. Blackston, J.A. Kable, C.D. Coles, M.E. Meeks.* Div Med Gen, Dept Pediatrics, Emory Univ Sch Medicine, Atlanta, GA.

Initial descriptions of dysmorphia in FAS were based on Caucasian samples (Jones and Smith, 1973). However, this disorder has been identified more frequently among minority groups, particularly Native Americans and African-Americans. It has long been a concern that facial features characteristic of African-Americans may contribute an overdiagnosis. These features include lower nasal bridge, anteverted nares, wide mouth and dermal patterns. In this study, we report preliminary data from a large metropolitan comprehensive fetal alcohol clinic where clientele is almost equally African-Americans and Caucasians. All patients are evaluated for FAS using a standard dysmorphology checklist which yields a weighted score (PedScore) of alcohol-related dysmorphology. To ascertain whether specific features contribute to an overdiagnosis of FAS in African-Americans, both summary data and individual items were evaluated in 254 consecutive cases, of whom 99 met our criteria for FAS. Among all children applying for services, Caucasians had a mean PedScore of 11.38 while African-Americans had a mean of 15.029, ($F=10.91$, $p<.001$) indicating a significant difference between these groups. Items that discriminated the groups included, light for gestational age (GA), small head for GA, ptosis, low nasal bridge, anteverted nares, thin upper lip, small mandible, large mouth, unusual palmar creases and hypoplastic nails. Because these findings could be biased by a higher incidence of FAS among African-Americans, we also analyzed the subgroup ($n=99$) who were diagnosed with FAS (PedScore >15). Among this group, there was no ethnic difference in mean scores (Caucasians: 20.897; African-Americans: 21.457; $F=2.93$, $p=0.59$). These findings may be accounted for in two ways. First, the teratogenic effects of alcohol itself could well influence the morphology, and secondly the results may be influenced by using the full body PedScore checklist. These data suggest that: 1) There are ethnic differences in craniofacial features that may contribute to overdiagnosis of FAS among African-Americans; 2) Physical examination for FAS should not be limited to craniofacial features.

An Unusual Presentation of Cenani-Lenz Syndactyly in a Patient with Kabuki Syndrome. *A.M. Elliott¹, M.H. Reed^{2,4}, J.A. Evans^{1,3,4}, A.E. Chudley^{1,3,4}.* 1) Department of Biochemistry & Medical Genetics, University of Manitoba; 2) Department of Radiology; 3) Department of Pediatrics and Child Health; 4) Children's Hospital, Winnipeg, Manitoba, Canada.

Cenani-Lenz syndactyly (CLS) a proposed AR entity, is classically characterized by total digit syndactyly, metacarpal and carpal fusions, and in some cases, radio-ulnar synostosis. The feet may only show 2-3 cutaneous syndactyly. We report a 6-year-old male with an unusual presentation of CLS who was the only child born to his French Canadian mother and Swedish/Scottish father. Evaluation at 5 months revealed 3 malformed digits on each hand. Radiographically, there was hypoplasia of the phalanges of the thumbs. Metacarpals 1 and 2 were normal. The index fingers were abnormal, with a widened proximal phalanx (PP) and a medially placed delta phalanx. Distally, there were 2 irregular ossicles side by side. The right 3rd and 4th metacarpals were closely proximated and an abnormal digit articulated with their heads. The PP of this digit was wide with 2 "kissing delta phalanges." The middle phalanx of this digit was wide, and the distal phalanx was reasonably normal. The 4th metacarpal was abnormally shaped. On the right, there was another relatively normal medial digit. On the left, in addition to the 5th digit, there was a crossbone that articulated with the medial aspect of the 4th metacarpal. Subsequent analysis revealed carpal coalition and posterior subluxation of the radial heads. The feet showed 2-3 cutaneous syndactyly. Renal ultrasound was normal. Examination at 6 years revealed normal growth, the digital anomalies, oligodontia, and features suggestive of Kabuki syndrome including: large prominent ears, sparse medially flared and arched eyebrows, wide palpebral fissures, long eyelashes and persistent fingertip pads. He has had recurrent episodes of otitis media and pneumonia. Development has been normal, according to his mother. Although he does not show the typical spoon hand configuration, he resembles other reported CLS patients. CLS should be considered in patients presenting with an unusual syndactyly/oligodactyly. The findings of CLS and Kabuki Syndrome in this child may be coincidental.

Siblings with Extreme Short Stature: The Differential Diagnosis of Primordial Dwarfism. *C. Flora¹, J. Neidich^{1,2}.*
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Primordial dwarfisms are a group of syndromes characterized by extreme short stature with microcephaly. Patients with osteodysplastic types of primordial dwarfism have a variety of skeletal and other anomalies. Some individuals in published reports were diagnosed with Seckel syndrome, but may have other types of primordial dwarfism. The delineation of these syndromes has been difficult due to heterogeneity and syndrome rarity.

The patients are two siblings with extreme short stature who are the offspring of first cousins. The elder, a female, was 32 months old at exam. Height was 50-centile for 8 1/2 months, weight for 4 1/2 months and OFC for 4 months. Bone age was significantly delayed. Developmental milestones were within normal limits. Her similarly affected brother was 12 months at exam. His growth parameters were height for 1 1/2 months, weight for 5 weeks, and OFC for 1 month. Both were growth retarded premature infants without other health problems or internal anomalies. They both had high-pitched voices, and were bilingual in English and Arabic.

The literature on primordial dwarfs and Seckel syndrome predominantly consists of case reports. Geneticists have suggested three types of osteodysplastic primordial dwarfism, in addition to Seckel syndrome and Silver-Russell syndrome. Most reports from the old literature do not establish that the individuals did not have a form of pituitary dwarfism. We review the literature, and present the likely diagnosis for these siblings.

Mosaic Monosomy 14- Clinical features and recognisable facies. *V.P.M. McConnell, D. McManus, P.J. Morrison.*
Department of Genetics, Belfast City Hospital Trust, Belfast, Co Antrim, Northern Ireland.

Monosomy 14 has not been described previously. Only 2 case reports of mosaic monosomy 14, in an Arab child with a low level (10%) of mosaicism and a Japanese child where the level of mosaicism was not indicated, have been described. We report a 1 year old child with severe mosaic monosomy 14 with up to 30% mosaicism. Prenatally ultrasound revealed ascites with cerebral ventricular dilatation and a left ovarian cyst with resolution of the ascites occurring post oophorectomy. Amniotic fluid analysis demonstrated complete monosomy of chromosome 14 in 31% of cells with the remaining 69% cells having a ring 14 chromosome. This result was confirmed on blood lymphocyte analysis post delivery. Clinical examination revealed facial dysmorphism including microcephaly and dolichocephaly, bilateral lower segment iris colobomas and retinal pigmentation, severe eczema and curly blond hair. Hypotonia with severe mental retardation and severe seizures were also present. This case demonstrates several of the abnormalities found in r14 and interstitial 14q deletions. In addition, several of the features are accounted for by loss of distal 14q, and the severe mental retardation has been attributed to the monosomy. This combination of 31% mosaic monosomy 14 / ring 14 appears to be the most severe phenotype described in a living case to date. Chromosome 14 appears to be important for craniofacial and skin development. The mental deterioration is severe and is compounded by the uncontrollable seizures.

Lethal bone dysplasia in spontaneous abortion fetus. A new non-described entity. *V. Moran¹, C. Ortiz-Hidalgo^{1,2}, I. Garcia-Pelaez^{1,3,4}, L. Cornejo⁴, E. Monterrubio¹, O. Aguirre⁴, H. Perez-Pineda^{1,4}, M. Arteaga^{1,3,4}.* 1) School of Medicine, Universidad Panamericana; 2) ABC Hospital; 3) Faculty of Medicine, National Autonomous University of Mexico; 4) Hospital Infantil de Mexico Federico Gomez, Mexico City, Mexico.

Introduction. Bone dysplasias are a complex group of disorders of difficult classification, with phenotypes that many times overlap, the difficulty is increased in affected fetuses, that may just resemble the phenotype in postnatal stages or even be lethal. **Case report.** A 18 wks gestation female fetus was obtained from a spontaneous abortion. The parents were non-consanguineous and there was no exposition to teratogens. The fetus presented generalized edema, head and body disproportion, increased cephalic perimeter, low-set ears, depressed nasal bridge, anteverted nostrils, elonged philtrum, arched mouth, very thick lips, microretrognathia, short neck, narrow torax, generalized shortening of limbs mainly rhizomelic and very thick skin. Karyotype analysis could not be performed. The necropsy showed an anomalous pulmonary venous connection, probably infradiaphragmatic. Histopathologic analysis showed thickening of the skin at expense of both edema and increased deposit of mucopolysaccharides; the femur had an abnormal growth plate with irregular proliferative zone; in other bones increased osteoclast activity was observed. **Discussion.** This case sheared representative characteristics of several diagnosis but did not full-filled a specific one. We considered Chondrodysplasia punctata because the rhizomelic shortening and the growth plate abnormalities as seen in this case, however our case lacked typical data in the radiological analysis. Another considered diagnosis was Geleophysic dysplasia because the similarity of the skin mucopolysaccharides deposits in facial area however, in our case the deposit was generalized, neither had the typical phenotype. **Conclusions.** We consider this case as a bone dysplasia of the Geleophysic or Chondrodysplasia punctata varieties, with more severe afectation, because of the lethal consequences, with the unusual characteristics described above, that probably had not been described before.

Marfanoid habitus with abnormal situs: A new syndrome? *K. Kosaki*¹, *L. Bird*², *R. Kosaki*³, *J. Maeda*¹, *M. Jones*², *M. Matsumoto*⁴. 1) Dept Pediatrics, Keio Univ, Tokyo, Japan; 2) Dept Dysmorphology, San Diego Children's Hosp, San Diego, CA; 3) Div Med Genet, Saitama Children's Hosp, Itwatsuki, Japan; 4) Dept Orthopedics, Keio Univ, Tokyo, Japan.

Marfanoid habitus suggests abnormal microfibril formation, whereas a situs ambiguus or situs inversus phenotype points to defective left-right axis determination. The concurrence of these two abnormalities had not been known until recently, when Gokce et al. reported a 22-year-old female with Marfan syndrome-like body habitus and situs inversus. We report two cases of a similar combination in unrelated patients. Case 1: A Japanese male was diagnosed as having valvular pulmonary stenosis, dextrocardia, azygous connection, and polysplenia during newborn period. At 14 years of age, he had decreased upper-lower segment ratio, large ears, pectus carinatum, scoliosis, hyperextensible fingers, arachnodactyly, and a positive wrist sign. Ophthalmologic examination revealed myopia, but there was no subluxation of the lenses. A skeletal survey revealed mild osteoporosis. The chromosomes were normal. Case 2: A Caucasian female was diagnosed as having situs inversus. At 11 years of age, the patient had decreased upper-lower segmentation ratio, large ears, scoliosis, hyperextensible fingers, arachnodactyly, and a positive wrist sign. Echocardiography showed dilatation of the aortic root. A skeletal survey revealed mild osteoporosis. Documentation of this specific combination of marfanoid habitus and abnormal situs in two additional patients gives further credence to the concept that the combination may represent a previously unrecognized syndrome.

Arterial aneurysms in two unrelated individuals with Sotos syndrome. *T. Jewett, L. Terry.* Dept. of Pediatrics, Div. of Medical Genetics, Wake Forest Univ. Health Sciences, Winston-Salem, NC.

Sotos syndrome, sometimes referred to as "cerebral gigantism," is an overgrowth syndrome characterized by birth weight and length greater than the 90th percentile, excessive linear growth in the first years of life, advanced bone age, and an unusual facies. Dysmorphic features include macrodolichocephaly, downslanting palpebral fissures, and a prominent, pointed chin. Most cases are sporadic, although autosomal dominant inheritance is suggested in some families. Affected children typically manifest speech and motor delays in early life, but intelligence is usually normal. There is an increased risk for neoplasia in Sotos syndrome of ~4%; otherwise, affected children are generally healthy. We report two, unrelated individuals with Sotos syndrome and arterial aneurysms of unknown cause, an association not previously described.

K.I. is a 9 y.o. boy who was diagnosed with Sotos syndrome at one year of age by a medical geneticist. Aside from Horner syndrome involving the left eye from early childhood, no neurologic deficits were noted. Intelligence is normal. At age 7, he presented with a one day history of progressive weakness and incoordination. Head imaging studies showed right cerebellar and bilateral brain stem infarcts, and angiography showed abnormal tortuosity and ectasia of the internal carotid arteries and multiple cerebral vessels, with dissection and thrombi in some arteries. He is being treated with anticoagulation.

R.B. is a 22 y.o. man who was diagnosed with Sotos syndrome by a medical geneticist at 8 years of age. He is mildly mentally retarded. At age 21, he presented to the emergency department complaining of exertional chest pain for one month. Cardiac catheterization showed a reduced ejection fraction, enlargement of the left ventricle, and decreased heart motion with aneurysms of the proximal left anterior descending, left circumflex, and right coronary arteries with thrombus formation. The possibility of past Kawasaki disease was questioned, but there is no history to support this. He has undergone coronary artery bypass and graft.

Mutation analysis of TBX1, HOXA3, HOXB3, and HOXD3 in patients with CHARGE association. *N. Yahagi¹, R. Kosaki², T. Suzuki¹, S. Lalani³, T. Takahashi¹, J. Belmont³, K. Kosaki¹.* 1) Pediatrics, Keio Univ, Tokyo, Japan; 2) Dept of Med Genetics, Saitama Children's Med Ctr, Iwatsuki, Japan; 3) Dept of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

The CHARGE association represents a nonrandom clustering of ocular coloboma, heart malformation, choanal atresia, retarded growth and development, central nervous system malformations, genital hypoplasia, ear abnormalities, or deafness. Based on anatomical distribution of major defects in CHARGE association, abnormal development of tissues derived from neural crest is implicated. Although pathogenesis of the CHARGE association is unknown, recurrence in several families points to contribution of genetic factors. Several patients with chromosomal abnormalities have been reported including deletion of 22q11. Because mice with loss-of-function mutation in *Tbx1* exhibit phenotype similar to CHARGE association and human *TBX1* resides within the interval commonly deleted in the 22q deletion syndrome, *TBX1* was considered as a promising candidate. In addition, because mice with loss-of-function mutation in *Hoxa3* leads to neural crestopathy, *HOXA3* was analyzed together with paralogous *HOX* genes *HOXB3* and *HOXD3* which presumably play complementary roles with *HOXA3*. Nineteen classic cases of CHARGE association were screened for mutations in using direct sequencing of PCR products amplified from genomic DNA. No pathogenic mutations were found in any of the genes. A novel Threonine to Proline amino acid change, which was found in both patients and normal controls, was considered as polymorphism. We conclude that mutation of the *HOXA3*, *B3*, *D3* and *TBX1* are not a common cause of the CHARGE association.

Three cases of Leber congenital amaurosis with an Ehlers-Danlos-like syndrome. *C. Yasutsugu¹, K. Naritomi², S. Takaesu³, T. Ohta¹.* 1) Dept Pediatrics, Univ Ryukyus Sch Medicine, Okinawa, Japan; 2) Dept Medical Genetics, Univ Ryukyus Sch Medicine, Okinawa, Japan; 3) Dept Ophthalmology, Univ Ryukyus Sch Medicine, Okinawa, Japan.

Leber congenital amaurosis (LCA) is the earliest and the most severe form of all inherited retinal dystrophies. Autosomal recessive inheritance is suspected in most LCA families. LCA is clinically and genetically heterogeneous and often associated with systemic anomalies. We report on three patients in two nonconsanguineous families, who had weak or no visual acuity, pigmentary retinal degeneration, nonrecordable electroretinograms on ophthalmological examinations in all cases, mental retardation, inability to walk with ataxia, characterized facies with bitemporal hollowing, high nasal bridge, thin upper lip, prominent chin, skeletal anomalies such as hyperextensible joints, cigarette-paper scar in one, odontoid instability in one, cerebellar vermis hypoplasia in one, growth hormone deficiency in one. The serum amino acid and blood gas analyses and the pyruvate and lactate levels in blood were all normal. This is the second report on Leber congenital amaurosis with an Ehlers-Danlos-like syndrome.

Development of skull dysmorphology in Ts65Dn segmentally trisomic mice. *J.T. Richtsmeier^{1,2,4}, J. Leszl^{1,2}, C.A. Hill¹, V. Aquino³, R.H. Reeves³*. 1) Department of Anthropology, The Pennsylvania State University, University Park, PA; 2) Program in Genetics, The Pennsylvania State University, University Park, PA; 3) Department of Physiology, The Johns Hopkins University, School of Medicine, Baltimore, MD; 4) Center for Craniofacial Development and Disorders, The Johns Hopkins University, School of Medicine, Baltimore, MD.

Down syndrome (DS) is the most frequent live-born aneuploidy and is caused by trisomy for human Chromosome (Chr) 21. DS results in a characteristic spectrum of developmental anomalies including effects on craniofacial tissues. The facial appearance in DS is highly characteristic, however, quantitative analysis demonstrates a marked variability in phenotypic expression among DS individuals. The increased variability noted in the DS population is argued to be a result of developmental instability, a process by which dosage imbalance for large numbers of genes disrupts multiple genetic pathways, resulting in developmental anomalies. The Ts65Dn mouse has segmental trisomy for mouse Chr 16 and is at dosage imbalance for many of the same genes triplicated in DS. We have previously quantified specific, completely penetrant skull phenotypes in adult Ts65Dn mice that parallel those defined for humans with DS. These skull phenotypes are caused by trisomy of the same genes triplicated in DS. We examine developmental instability of the Ts65Dn mouse by analyzing fluctuating asymmetry of skull features, and present the results of a morphometric analysis of the skulls of P0 Ts65Dn trisomic mice and their euploid littermates. Our analysis of fluctuating asymmetry reveals that developmental instability contributes to the documented skull dysmorphology in adult Ts65Dn mice. Our morphometric analysis demonstrates that the skull dysmorphology is already established at birth. The disruption of development that causes the skull phenotypes occurs prenatally, affects the size of all skull elements, and produces localized changes in shape of specific skull regions.

A complex autosomal dominant syndactyly type-I in a very large Indian family. *U.C. Rao¹, M. Raveendrababu¹, M.R. Memon², J.V. Solanki³, U. Ratnamala¹, U. Radhakrishna¹.* 1) GeneHealth, Green Cross Blood Bank & Genetics Centre, Ahmedabad; 2) Department of Zoology, School of Sciences, Gujarat University Ahmedabad; 3) Department of Animal Genetics & Breeding, College of Veterinary Science & Animal Husbandry, Gujarat Agricultural University, Anand-388001, India.

Syndactyly is the most common malformation of the hand with a frequency of 1:2000 to 1:3000 live births. It was classified into five different types. Syndactyly type-I (SDTY1) (OMIM 185900) involves complete or partial bilateral syndactyly between third and fifth fingers which is occasionally associated with fusion of the distal phalanges. Feet are rarely affected. It may be an isolated condition. The genes responsible for SDTY1 and SDTY2 have been mapped to chromosome 2q34-q36 (Am J Hum Genet 67:492-97, 2000; Am J Med Genet 104:147-151, 2001 and 2q31 (Hum Molec Genet 4:1453-1458,1995), respectively. We have studied a large six generation Indian pedigree with an autosomal dominant SDTY1. The pedigree consists of 645 individuals including 290 affecteds (127 males/163 females). The anomaly was present in both extremities and the phenotype appeared to be 100% penetrant. The expression of the phenotype was quite variable and ranged from bilaterally affected with typical features of SDTY1 affecting the 3rd and 4th fingers to unilateral, bilateral hand syndactyly and unilateral partial syndactyly of 2nd and 3rd toes, unilateral elongation of 2nd and 3rd toes with complete soft tissue syndactyly of all fingers, complete ectrodactyly involving toes, reduction in finger size, nail syndactyly, duplication of nails. Unilateral Polydactyly of 3rd finger with duplication of 5th toe was observed in two patients. The degree of severity was very high when both parents were affected with SDTY1. These include ectrodactyly of hand, soft tissue syndyctyly of all fingers and toes, hypoplastic nails, absent nails, club shaped hand and foot. Distortion of dermatoglyphic patterns was also observed. Linkage studies with markers closely linked to SDTY1 will both confirm allelism to this locus and may reduce the genetic interval encompassing the SDTY1 gene or provide evidence for genetic heterogeneity. u_c_rao@hotmail.com.

A TDT and Candidate Gene Analysis demonstrates a role for genes of folate metabolism (RFC1, MTHFR) in clefting. *D.M. Caprau¹, J.C. Murray¹, P.A. Romitti², R. Grady¹.* 1) Dept of Pediatrics, Univ of Iowa, Iowa City, IA; 2) Dept of Epidemiology, Univ of Iowa, Iowa City, Iowa.

Cleft lip and cleft palate are among the most common birth defects. Each has a complex etiology involving genetic and environmental factors. Candidate gene analysis has previously suggested both MSX1 and TGFB3 are risk factors for clefting in Caucasian populations. This study looked at triads (mother, father and affected proband). The objective of this study is to evaluate the role of selected candidate genes in the etiology of CL/P from a predominantly Caucasian population born in Iowa. The genes selected are: MTHFR, GCP II, RFC1 (folate metabolism), MSX1 (transcription) and TGFB3, TGFA, PVRL2 (signaling molecules). These genes were selected based on their expression in the developing palate, phenotype in knockout mice or from their role in gene-nutrient interactions. Patients were evaluated by pediatricians and clinical geneticists for phenotype classification. DNA was extracted from whole blood or buccal swabs. All samples were genotyped for at least one polymorphism in each gene using conventional or kinetic PCR. A TDT was used to look for evidence of transmission distortion. 261 triads were genotyped for all markers. Significant p-values on the TDT test were found for over transmission of the A allele for RFC1 G80A marker ($p=0.0001$). The C allele for MTHFR C677T was also over transmitted with CL/P ($p=0.02$). This study carried out an extensive search for evidence of transmission distortion in 7 candidate genes of high priority for the study of cleft lip and palate. Significant p-values found for RFC1 and MTHFR suggests a strong role for folate metabolism contributing to clefting. Other studies of human birth defects, especially NTDs, have also supported a role for folate deficiency in etiology. The role of RFC1 and MTHFR suggests that a subpopulation of individuals may have a genetically increased risk for clefts. Such groups may benefit from more targeted and/or higher doses of folate for prevention of recurrences or occurrences.

Toward a Genetic Etiology of CHARGE Syndrome. *S.R. Lalani¹, D.W. Stockton¹, C. Bacino¹, L.M. Molinari¹, N.L. Glass^{2,3}, S.D. Fernbach¹, J.A. Towbin^{1,3,4}, W.J. Craigen¹, J.M. Graham, Jr.⁶, M.A. Hefner⁷, A.E. Lin⁸, K.L. McBride¹, S.L. Davenport⁵, D.M. Martin⁹*. 1) Dept of Molecular and Human Genetics; 2) Dept of Anesthesiology; 3) Dept of Pediatrics; 4) Dept of Pediatric Cardiology, Baylor College of Medicine, Houston, TX; 5) Sensory Genetics/ Neuro-Development; 6) Cedars Sinai Medical Center, UCLA Medical School, Los Angeles, CA; 7) Dept of Pediatrics, St. Louis University, MO; 8) Genetics and Teratology Unit, MA; 9) Depts of Pediatrics and Human Genetics, University of Michigan, MI.

CHARGE syndrome is characterized by ocular coloboma, cranial nerve abnormalities, distinctive heart defects, choanal atresia, retardation of growth and development, genital hypoplasia, ear abnormalities and/or hearing loss. While clinical delineation of this syndrome has progressed, the molecular basis for the disorder remains unknown. We hypothesized that CHARGE is caused by genomic microdeletion(s) and performed genotyping in ten case/parent trios to detect potential loss of heterozygosity. Using 811 microsatellite markers of 5 cM average spacing from the ABI Prism Linkage Mapping set-HD5 v.2, eight markers showed a persistent non-Mendelian pattern of inheritance in 1/10 to 3/10 families. BAC and PAC clones containing the sequence of these markers, *DIS2833*, *D5S417*, *D6S434*, *D7S2560*, *D9S271*, *D12S1646*, *D19S420* and *D20S178* were used to perform fluorescence *in situ* hybridization, but no deletion was observed at these loci. Interestingly, *D12S1646* maps within the *TBX5* gene. Southern blot was performed using a cDNA probe, which excluded a deletion of the *TBX5* gene in the proband. Using this experimental design, approximately 40% of the genome has been ruled out for a 2 Mb deletion in 10 patients with CHARGE syndrome. Efforts are also underway to identify chromosomal breakpoints in a patient with CHARGE syndrome described by Martin et al. (AJMG 99:115-9) with an apparently balanced translocation t(2;7)(p14;q21.11). This patient offers a unique opportunity to identify genes that may be involved in critical developmental pathways in CHARGE syndrome.

Diencephalic tumour-emaciation syndrome X Lipoatrophies: differential diagnosis. *C.A. Kim, L.M. Pinto, A.C. Paula, L.M.J. Albano, A.L.F. Tupinamba, M.G. Vallada, H.S. Marques, E. Vidolin, Y.K.L. Koda, D.R. Bertola.* Dept Pediatrics, Inst da Crianca, Sao Paulo, Brazil.

The diencephalic tumour-emaciation syndrome of infancy, first described by Russell (1950), is caused by a slowly growing glioma in the anterior hypothalamus, usually an astrocytoma. It is characterized by a progressive emaciation in spite of good food intake, undiminished alertness, pallor without anemia, occasional vomiting and nystagmus. No neurological symptoms and signs could be detected for a long time, causing delay in its diagnosis. Usually, its onset is precocious, varying from early infancy to the end of the second year of life. We report on a 1y7mo girl with this syndrome in order to emphasize its differential diagnosis with lipodystrophy. She was the first child from a healthy and nonconsanguineous couple. Her birth weight was 3,800 g and length, 49 cm. She was normal until 6 months of age, when she presented severe and progressive failure to thrive in spite of a good food intake and sporadic diarrheas. Physical exam at 1y7mo showed an active and alert girl, with a weight of 6,300g ($p < 2,5$); height of 76 cm ($p < 2,5$); and OFC of 46cm ($p = 50$); normal hair, generalized absence of adipose tissue, without muscular hypertrophy or hepatomegaly. Laboratorial findings showed normal values of glucose tolerance test, lipaemia, absorption tests. Abdominal ultrasonography was also normal. Ophthalmologic funduscopy revealed a pallor and partial atrophy of optic nerves. CT scan and MRI images showed a hypophalamic tumour lesion with ventricles enlargement. She was submitted to a partial resection (40%) of the tumor and currently, under chemotherapy. We recommend the investigation of diencephalic tumors in other syndromes that share the emaciation signs, such as leprechaunism, lipodystrophies, Cockayne and other progeroids syndromes, once a prompt diagnosis of this tumor can improve its clinical course and survival.

A twin study of ADHD. *M.B. Strassberg*¹, *L. Murrelle*^{2, 3}, *L. Corey*^{2, 3}, *M.L. Marazita*^{4, 5, 6}, *B.S. Maher*^{4, 5}, *M.M. Vanyukov*^{4, 7}. 1) University of Texas, Houston Health Science Center, Houston, TX; 2) Mid-Atlantic Twin Registry, Virginia Commonwealth University, Richmond, VA; 3) Virginia Institute for Psychiatric and Behavioral Genetics, Virginia, Commonwealth University, Richmond, VA; 4) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 5) Cleft Palate-Craniofacial Center, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA; 6) Department of Oral and Maxillofacial Surgery, University of Pittsburgh, Pittsburgh, PA; 7) Center for Education and Drug Abuse Research (CEDAR), Department of Pharmaceutical Sciences, University of Pittsburgh, Pittsburgh, PA.

Attention Deficit/ Hyperactivity Disorder (ADHD) is a significant public health problem. The main purpose of the present study was to evaluate the heritability of liability to ADHD and its dimensions in preschool aged children. This study used model-fitting to estimate genetic and environmental contributions to the variation in the core behavioral dimensions associated with ADHD in two sets of young twins, aged 1 -5 years. Data from the CBCL 1 -5 years was obtained for 21 pairs of monozygotic (MZ) twins and 21 pairs of dizygotic (DZ) twins enrolled in the Pittsburgh Registry of Twin Multiplets (PRIM). Data from the Young Twins Survey was obtained for 101 pairs of MZ twins and 147 pairs of DZ twins enrolled in the Mid-Atlantic Twin Registry (MATR). Univariate analyses supported a substantial contribution of genetic factors in the expression of inattention, impulsivity/hyperactivity and ADHD problems in males, and a lesser contribution of genetic factors involved in the expression of these traits in females. Bivariate analyses indicated that the correlation between attention and hyperactivity/impulsivity is genetically mediated.

Conclusion: Genetic factors are important in variation in the expression of the separate dimensions of ADHD and in the covariation between them in young children. The extent of genetic effect differs between males and females.

Exclusion of candidate loci and autozygosity mapping of Jeune asphyxiating thoracic dystrophy. *N.V. Morgan¹, C. Bacchelli², M. Silengo³, J.H. Tuerlings⁴, L.C. Wilson⁵, L.A. Brueton⁶, E.R. Maher¹, R.C. Trembath⁷, P.J. Scambler², F.R. Goodman², C.A. Johnson¹.* 1) Medical and Molecular Genetics, University of Birmingham, Birmingham, W. Midlands, UK; 2) Molecular Medicine Unit, Institute of Child Health, London, UK; 3) Dipartimento di Scienze Pediatriche e dell'Adolescenza, Università degli Studi di Torino, Torino, Italy; 4) Department of Human Genetics, University Hospital Nijmegen, Nijmegen, The Netherlands; 5) Department of Clinical & Molecular Genetics, Institute of Child Health, London, UK; 6) Clinical Genetics Unit, Birmingham Womens Hospital, Birmingham, UK; 7) Division of Medical Genetics, University of Leicester, Leicester, UK.

Jeune asphyxiating thoracic dystrophy (JATD; MIM No 208500) is a rare recessively-inherited malformation syndrome characterised by both skeletal and extra-skeletal involvement. Skeletal abnormalities include a small narrow thorax, shortening of the ribs and long bones, metaphyseal irregularities and occasional postaxial polydactyly. Renal, hepatic, pancreatic and retinal abnormalities may also occur. Severely affected infants often die in the neonatal period from respiratory insufficiency, and chronic renal failure is common in those patients who survive. The molecular basis of JATD is at present unknown. A similar phenotype occurs in Ellis-van Creveld syndrome, and has been reported in one case with a de novo deletion of 12p11-p12. JATD may therefore result from mutations in the EVC gene, or in a gene located on chromosome 12p. A possible mouse model of JATD, the shorty (srt) mutant, has also recently been identified through an ENU mutagenesis screen. We have ascertained 6 consanguineous JATD families (3 from Pakistan, 2 from Italy and 1 from Holland) for linkage studies. 2 families have 2 affected children. In all 6 families, haplotype analysis has excluded linkage to the EVC gene, to chromosome 12p, and to the chromosomal regions that are syntenic to the 7 cM interval on mouse chromosome 17 to which srt has been mapped. To identify loci linked to JATD, we have therefore adopted an autozygosity mapping approach and are currently carrying out a genome-wide linkage screen of the 8 affected children.

A novel locus for Meckel-Gruber syndrome, MKS3, maps to chromosome 8q24. *C.A. Johnson¹, N.V. Morgan¹, P. Gissen^{1,2}, S. Malik Sharif³, L. Baumber⁴, J. Sutherland⁴, D.A Kelly², K. Aminu⁴, C.P. Bennett⁵, C.G. Woods³, R.F. Mueller^{3,5}, R.C. Trembath⁴, E.R. Maher¹.* 1) Medical & Molecular Genetics, University of Birmingham, Birmingham, W. Midlands, UK; 2) Children's Liver Unit, Princess of Wales Children's Hospital, Steelhouse Lane, Birmingham, UK; 3) Department of Clinical Genetics, Yorkshire Regional Genetics Service, St. James's University Hospital, Beckett Street, Leeds, UK; 4) Division of Medical Genetics, Departments of Medicine and Genetics, University of Leicester, Leicester, UK; 5) Molecular Medicine Unit, University of Leeds, St. James's University Hospital, Leeds, UK.

Meckel-Gruber syndrome (MKS), the most common monogenic cause of neural tube defects, is an autosomal recessive disorder characterized by a combination of renal cysts and variably associated features, including developmental anomalies of the central nervous system (typically encephalcoele), hepatic ductal dysplasia and cysts, and polydactyly. Locus heterogeneity has been demonstrated by the mapping of the MKS1 locus to 17q21-24 in Finnish kindreds, and of MKS2 to 11q13 in North African-Middle Eastern cohorts. In the present study, we have investigated the genetic basis of MKS in eight consanguineous kindreds, originating from the Indian subcontinent, that do not show linkage to either MKS1 or MKS2. We report the localization of a third MKS locus (MKS3) to chromosome 8q24 in this cohort by a genome-wide linkage search using autozygosity mapping. We identified a 26 cM region of autozygosity between D8S586 and D8S1108 with a maximum two-point LOD score at D8S1179. A heterogeneity test provided evidence of one unlinked family. Exclusion of this family from multipoint analysis maximized the multipoint LOD score at locus D8S1128 ($Z_{\max} = 5.65$). Furthermore, a heterozygous SNP in DDEF1, a putative candidate gene, suggested that MKS3 mapped within a 15 cM interval. Comparison of the clinical features of MKS3-linked cases with reports of MKS1- and MKS2-linked kindreds suggests that polydactyly (and possibly encephalocele) appear less common in MKS3-linked families.

Familial Venous-occlusive disease of the liver with immunodeficiency: homozygosity mapping and call for patients.

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We present three families of Middle Eastern origin (Mellis et al, 1976, Wong et al., in review) with an apparently heritable form of venous-occlusive disease of the liver with immunodeficiency (VODI) requiring treatment with intravenous immunoglobulin. Multiple consanguinity in two families with common ethnic origins suggests the presence of a causative autosomal recessive gene. Familial forms of this condition have not appeared in the literature subsequent to the initial reports. These families therefore represent a unique resource to aid in the genetic mapping of VODI. Venous-occlusive disease of the liver (VODL) consists of fibrous concentric narrowing/obliteration of zone 3 terminal hepatic venules with subsequent damage to centrilobular hepatocytes (Shulman et al., 1980, Valla et al., 1991). This histological appearance was first described in VODL secondary to ingestion of beverages containing pyrrolizidine alkaloids (Selzer et al., 1951). VODL has subsequently been reported with the ingestion of alcohol, toxins, the oral contraceptive, antineoplastic medications and hepatic irradiation. VODL is now most often seen within twenty days of a post bone marrow transplant. This cohort of consanguineous families with a common ethnic origin allows the opportunity to identify regions of homozygosity in affected individuals via homozygosity mapping. Identification of such regions may allow a candidate gene approach to identify a genetic aetiology for VODI. Identification of the genetic basis for VODI could lead to therapies for a life threatening complication of bone marrow transplantation. A genome wide scan is currently in progress.

***SMADIP1* is not a major gene in isolated agenesis of the corpus callosum.** *Y. Espinosa-Parrilla, F. Encha-Razavi, T. Atti-Bitach, P. Gosset, A. Munnich, M. Vekemans, S. Lyonnet, J. Amiel.* Department of Genetics, INSERM U-393. Hpital Necker-Enfants Malades, Paris, France.

Mutations of the smad binding protein 1 gene (*SMADIP1*, MIM 605802) result in a polytopic embryonic defect (Lurie-Mowat syndrome, MIM 235730), including facial dysmorphism, mental retardation, epilepsy and postnatal microcephaly as consistent features. However, frequent additional abnormalities include enteric nervous system malformation (Hirschsprung disease), agenesis of the corpus callosum (ACC), cardiac defects, renal abnormalities, and hypospadias. Among this broad spectrum of malformations ascribed to *SMADIP1* haploinsufficiency, ACC is the only feature that can be detected antenatally. In order to investigate whether the *SMADIP1* gene may be a major gene in ACC, we searched for *SMADIP1* deletions or mutations in a total of 8 fetuses collected from legally terminated pregnancies (16-36 weeks of gestation) after diagnosis of ACC by antenatal echography survey and normal chromosomal analyses (RTBG, 400 bands resolution). In addition, a significant proportion of cases presented cardiac defects (3/8) and/or urogenital anomalies (2/8). Screening for *SMADIP1* deletions was performed both by poly (CA) microsatellite markers flanking *SMADIP1* and by real-time semiquantitative PCR. Point mutation detection was performed by SSCP of the whole gene coding sequence. Neither deletions encompassing the *SMADIP1* gene, nor mutations could be detected. These results exclude the *SMADIP1* gene as a major gene in ACC.

Further molecular characterization of a boy with Sotos syndrome and partial trisomy of the long arm of chromosome 7. B.A. Fernandez¹, L. Penney¹, C. Prasad², S. Moore¹, I. Teshima³, K. Nakabayashi³, S.W. Scherer³. 1) Discipline of Genetics, Faculty of Medicine, Memorial University of Newfoundland, St John's, NF, Canada; 2) Dept of Child Health and Pediatrics, Childrens Hospital, Winnipeg, MB, Canada; 3) Dept of Genetics, The Hospital for Sick Children, Toronto, ON, Canada.

Sotos syndrome (OMIM # 117550) is characterized by overgrowth, advanced bone age, developmental delay and particular facial features. Most cases are sporadic, although autosomal dominant pedigrees have been reported. Recently *NSDI* was isolated from the 5q35 breakpoint of an individual with Sotos syndrome. Haploinsufficiency of *NSDI* appears to be a major cause of the syndrome, but genetic heterogeneity is believed to exist. A limited number of affected individuals with other cytogenetic abnormalities have been described; none of these has involved chromosome 7.

We report a 16 year-old boy with Sotos syndrome and an unbalanced karyotype. Bilateral cryptorchidism was noted at birth, and he was large for gestational age. He now has macrocephaly, with height at the 95th percentile and weight at the 75th percentile. He has global developmental delay, and functions at an 8-year level. His features include frontoparietal balding, a prominent chin, and disproportionately large hands. Brain MRI showed mild cerebral ventriculomegaly. DNA testing for Fragile X syndrome was negative, as was his family history.

Routine and spectral karyotyping revealed a *de novo* unbalanced karyotype with partial trisomy of 7q [46,XYder(14)t(7;14)(q32~34;p12~13)]. FISH studies delineated a trisomic segment extending from 7q35-qter. BACs from 7q35 (NH413B12 and CIT2572A14), as well as a BAC containing *D7S427* from 7q36, all showed trisomic signals. STR analysis to determine the parent-of-origin of the trisomic segment are in progress, and the *NSDI* locus will be examined to determine whether or not it is intact. The association of Sotos syndrome with partial trisomy of 7q suggests that this syndrome may sometimes be due to a dosage effect of one or more genes from this region.

Exclusion of the transcriptional repressor *SALL1* as a candidate gene in Branchio-oculo-facial syndrome. W.

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Branchio-oculo-facial syndrome (BOF; MIM 113620); is a rare autosomal dominant disorder. The symptoms of this disorder include bilateral postauricular cervical branchial sinus defects with hemangiomatous, scarred skin, cleft lip with or without cleft palate, pseudocleft of the upper lip, nasolacrimal duct obstruction, low set ears with posterior rotation, a malformed, asymmetric nose with broad bridge and flattened tip, and -occasionally- prematurely gray hair. Previous attempts to associate the phenotype with a candidate gene excluded the members 1-4 of the *EYA* (eyes abSENT) gene family. It was also shown that the similar branchio-otic and branchio-oto-renal syndromes are not allelic with BOFS. Recent findings about the expression of *SALL1* in the developing brain and the limbs suggested that this transcriptional repressor might be a candidate gene for BOF syndrome. *SALL1* is the gene mutated in Townes-Brocks syndrome (MIM: 107480), another syndrome with sensorineural hearing loss, urogenital anomalies, and preauricular protuberances. The overlapping symptoms prompted a study of *SALL1* as a candidate gene for BOF syndrome. We studied the cosegregation of *SALL1* alleles with the phenotype in a three generation family with five affected members. This is the largest pedigree with BOF syndrome reported until now. For that purpose we analyzed highly polymorphic dinucleotide repeat markers in an interval of 26 Mbp on chromosome 16. In an interval of 7.2 Mbp, where *SALL1* maps, we excluded cosegregation of alleles with the phenotype and thus present evidence that *SALL1* is not a candidate gene for BOF syndrome.

PHENOTYPICAL ANALYSIS OF AN AFFECTED POPULATION WITH WAARDENBURG SYNDROME IN COLOMBIA. *M. Rodriguez, N. Gelvez, M.C. Lattig, M. Tamayo.* Instituto de Genetica Humana, Facultad de Medicina, Universidad Javeiana, Bogota, Colombia.

Waardenburg Syndrome (WS) is characterized by hypopigmentation (hair, eyes and skin), deafness, broad nasal root and dystopia canthorum. The lateral displacement of the inner canthi of the eyes, classify this syndrome in WS type 1 (presence of the dystopia) and WS type 2 (absent of the dystopia). It is an autosomal dominant disorder with remarkable clinical and genetic heterogeneity. The purpose of this study was to expand the clinical knowledge on Waardenburg Syndrome (WS). We evaluated 138 individuals with Waardenburg Syndrome (93 propositus and 45 affected relatives) and carry out ophthalmological, audiological and genetic evaluation, to establish the phenotypic variability in our Colombian affected population. The most frequent phenotypic feature among affected individuals, was sensorineural deafness (80.5%), followed by broad nasal root (60.1%), white forelock (31.8%), synophrys (28.2%), heterochromia irides (25.3%), skin hypopigmentation (23.1%), blue intense iris (13.7%), parpebral ptosis (12.3%), and premature gray (8.7%). Curiously, we found a very low frequency of refractive errors. With regards to the kind of deafness we defined a profound and bilateral sensorineural deafness in the 63.7%, unilateral sensorineural in the 5.9% and, asymmetrical sensorineural in the 10.9%. The remaining 19.5% had normal hearing. Neurological development was normal in the 86.8% and abnormal in the 13.2%. We emphasize that not all of the affected individuals showed a complete set of clinical findings for Waardenburg Syndrome. In our study, the propositus were found to have 2 or 3 typical characteristics, while their relatives of them presented only one. This fact, makes the diagnosis more difficult and remarks the necessity to improve a complete familial study in each affected individual. Our information about phenotypic characteristics of the syndrome, confirm intra and inter familial clinical variability, and the researcher must be aware of this fact in order to make a correct clinical diagnosis and give to patients an adequate genetic counseling.

Analysis of genes deleted in Williams syndrome based on measurement of gene copy number by real-time PCR.

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Williams syndrome (WS) is a contiguous gene syndrome with variable phenotypic expression caused by a submicroscopic deletion at 7q11.23 that involve several genes, including elastin(ELN), LIMK1, syntaxin 1A(STX1A), and FZD. Haploinsufficiency for these genes in the deletion causes the WS phenotype and variability in the phenotype could be due to the heterogeneity of size and location of the deletion breakpoints. We have investigated the size of the deletions and defined the deleted map of the critical region from 27 patients with classical WS. Conventional methods to detect the haploinsufficiency include southern blots, pulsed-field gel electrophoresis, fluorescence in situ hybridization and analysis of polymorphic markers. However these methodologies are time-consuming and labor-intensive. In this study, we used a real-time quantitative PCR assay to assess gene copy number for detection of haploinsufficiency on WS regions. Six genes which are CNDL4, FZD4, STX1A, ELN, LIMK1, and WBSCR23 were analyzed by using TaqMan probes specific to each genes and the comparative CT method. Twenty-four patients had a deletion beginning from CNDL4.

Molecular analysis of EEC and related syndromes: Rapp-Hodgkin syndrome is due to mutations in P63. I.

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Mutations in P63 have been identified in several malformative syndromes, most of which are characterized by ectodermal dysplasia. An apparent conclusive genotype-phenotype correlation has been observed. EEC (ectodermal dysplasia, ectrodactily, lip/palate cleft: MIM 604292) and split-hand/foot malformation (SHFM: MIM 183600) are caused by mutations affecting the DNA binding domain of the p63 protein, whereas ankyloblefaron-ectodermal dysplasia-cleft (AEC: MIM 106260) is caused by mutations affecting the SAM domain and limb-mammary syndrome (LM: MIM 603543) is caused by frameshift mutations in exon 13-14. The aim of our study has been to characterise three multiplex families: two had EEC, one had Rapp-Hodgkin syndrome (RH, cleft lip/palate and ectodermal dysplasia: MIM 129400). Segregation of 3q highly polymorphic markers (D3S3530 and D3S1294) showed concordant segregation with the disease in all the families. Causal mutations were found in the three families. Mutations showed concordant segregation with the disease in the three families. EEC families carried mutations in the DNA binding domain of p63 (R279C, R304Q). These mutations are recurrent mutations since they have been found in several unrelated families, so far. Our data show that RH syndrome is due by a defect in P63. The mutation, which is new, is located in exon 14 and is a frameshift mutation (1860DelA). All the frameshift mutations identified so far in this region were present in patients with limb-mammary syndrome. The patients in our family have normal nipples and the affected mother has breast-fed her children. The hypothesis of a conclusive genotype-phenotype correlation as previously reported has not been completely supported by our data.

Type II methemoglobinemia: a novel mutation in NADH-cytochrome b5 reductase. *M. Galdzicka¹, P.E. Newburger², L.A. Demmer², S. Patnala¹, J.F. Cai¹, M.G. Hirshman¹, E.I. Ginns¹.* 1) Brudnick Neuropsychiatric Research Institute, Dept. of Psychiatry, Univ. of Massachusetts Medical School, Worcester, MA; 2) Dept. of Pediatrics, Univ. of Massachusetts Medical School, Worcester, MA.

Autosomal recessive methemoglobinemia is characterized by a decreased activity of either NADH-Cytochrome b5 reductase (b5R; DIA1) or of cytochrome b5. In type I the enzyme deficiency is limited to red cells, while in type II it is present in all tissues and involves both the soluble and microsomal forms of the enzyme. Individuals with type I methemoglobinemia have clinical manifestations limited to their hematopoietic system, while those with type II methemoglobinemia have a severe course with profound mental retardation and progressive neurological manifestations. In this report, we describe the clinical and molecular findings in a girl with type II methemoglobinemia.

Baby girl HL presented with severe neonatal methemoglobinemia, with up to 25% methemoglobin in the first week of life. NADH-Cytochrome b5 reductase (b5R) activity was 1 IU/gram hemoglobin (normal range 6-12 IU/gram) in her peripheral blood erythrocytes at age 27days. At her present age of 11 months, she has chronic mild methemoglobinemia (9%). On examination she is severely developmentally delayed with microcephaly and hypotonia.

The gene for DIA1 is located on chromosome 22 and is 31kb in size. It contains 9 exons (1922bp) and 8 introns, encoding a protein of 301 amino acids. The 5'UTR of 58bp is in exon 1 and the 3'UTR of 958bp is in exon 9. Sequence analysis of all 9 exons and exon/intron junctions of gene in the patient and her mother revealed a novel mutation, deletion of a G in exon 5, that was homozygous and heterozygous in the child and mother, respectively. This deletion of G in exon 5 results in a stop codon in exon 6, truncating the enzyme at 177 amino acids. The mutation identified in our patient supports the previous suggestion that methemoglobinemia type II is more often associated with full stops or deletions in the gene, while in type I disease, missense mutations are more frequently observed.

Genotype Governs the Clinical Spectrum of Neuroserpinopathy. *F.L. Lacbawan*^{1,2}, *R.L. Davis*³, *G.H. Collins*³, *P. Kent*⁵, *P.D. Holohan*⁴, *A.E Shrimpton*³, *M. Muenke*², *D. Krasnewich*². 1) Medical Genetics, Children's Natl Med Ctr, GWU, Washington DC; 2) Medical Genetics Br, NHGRI, NIH Bethesda MD; 3) Pathology; 4) Pharmacology; 5) Neurology, SUNY Upstate Medical University, Syracuse NY.

Neuroserpin is a brain-associated inhibitor of tissue plasminogen activator involved in modulating cell migration, axon outgrowth and synaptic plasticity. After our report of the first mutations S49P and S52R in Familial Encephalopathy with Neuroserpin Inclusion Bodies (FENIB) (Davis et al., 1999a, 1999b), S52R (Takao, et al., 2000), G392E and H338R (Davis, et al., 2002) were reported. These mutations in the shutter region cause misfolding and accumulation of mutant neuroserpin (Collins bodies) in the neurons of the cortex and subcortical structures because of loop-sheet polymerization similar to the α -1-antitrypsin variants. The loss of inhibitory function of S49P mutant has been clearly demonstrated (Belorgey, et al., 2002). However, we have presented strong evidence that the inclusion-body formation is a sufficient cause of neurodegeneration as supported by the direct correlation of the rate and magnitude of protein aggregation and the onset and severity of the disorder (Davis, et al., 2002). As a consequence of the severe degree of molecular instability, G392E>H338R>S52R> S49P, the G392E patient died at 19 years of age. Multiple grape-like Collins bodies are noted and progressive myoclonic epilepsy (PME) presented as cognitive delay and generalized seizures initially at age 13 years. In contrast, review of medical records of the seven affected members of the original family with S49P revealed onset at the fifth decade of life with course of about 16 years. Collins bodies are rarely in clusters with dementia as the predominant manifestation with variable extrapyramidal and frontal lobe symptoms and seldom seizures. PME is more common in children and young adults as opposed to dementia in older adults. Despite some clinical heterogeneity that exists within the same genotype, the genotype still dictates the onset and severity of this conformational disease. Hence, it must be an essential differential diagnosis for patients of any age presenting with cognitive delay, seizures, myoclonus, and/or early-onset dementia.

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Friedreich's Ataxia mimicking Hereditary Motor and Sensory Neuropathy. *M. Panas, N. Kalfakis, G. Karadima, P. Davaki, D. Vassilopoulos.* Dept Neurology, Athens National University, Athens, Greece.

Four patients from three unrelated families, with clinical and electrophysiological findings compatible with the diagnosis of hereditary motor and sensory neuropathy, are presented. The molecular analysis showed that the affected individuals were homozygous for the mutation in the X25 gene, characteristic of Friedreich's ataxia. These patients seem to represent a form of Friedreich's ataxia mimicking Charcot-Marie-Tooth disease.

Sensitivity and Specificity of the clinical diagnosis of spinocerebellar ataxias compared to molecular diagnosis.

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The spinocerebellar ataxias are a clinically and genetically heterogeneous group of diseases, with 16 assigned loci. The diagnosis of an individual with ataxia is based on molecular testing, available for 9 SCAs: 1, 2, 3, 6, 7, 8, 10, 12 and 17. One of the difficulties encountered in a clinical setting is that the phenotypic overlap of the different SCAs often makes it necessary to perform the complete ataxia testing panel, implying an elevated cost that may be unaffordable for low-income populations, e.g. in Developing Countries. A more rational approach would be focused-testing based on a presumptive clinical diagnosis. We analyzed a cohort of 94 Mexican SCA patients: a presumptive clinical diagnosis was made by a blind observer and was later compared to the results of the molecular test. Clinical suspicion was helpful in the identification of SCA7, SCA10 and probably SCA17, but we were unsuccessful in the classification of SCA1, 2, 3, 6, 8 and 12. We suggest that a rational approach to molecular testing would be to test for SCA7, SCA10 or SCA17 when a strong clinical suspicion exists, and then to test according to the prevalence of each SCA in a given population.

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	SCA2	SCA3	SCA7	SCA10	SCA17
Sensitivity	0.647	0.333	0.900	0.810	0.750
Specificity	0.875	0.952	0.979	0.972	0.986
(+)PredVal	0.815	0.667	0.750	0.895	0.750
(-)PredVal	0.745	0.833	0.993	0.945	0.986

Phenotypic diversity in human malformation syndromes are modifier genes important? *A.M. Slavotinek*. Bldg 49, Rm 4B75, NHGRI/NIH, Bethesda, MD.

It has proven difficult to establish phenotype-genotype correlations for human malformation syndromes. The role of modifier genes in phenotypic variation has most clearly been established for metabolic diseases such as cystic fibrosis and phenylketonuria. Recently, complex inheritance patterns including modifier genes have been described for syndromes and malformations, including triallelic inheritance in Bardet-Biedl syndrome and oligogenic inheritance in Hirschsprung disease. It can therefore be hypothesized that modifier genes are underestimated as determinants of phenotypic variability in human malformation syndromes. Published case reports of two autosomal recessive syndromes were examined, one syndrome with a relatively specific phenotype (Robinow syndrome [RS]; 71 cases) and one showing greater phenotypic variation with heterozygote manifestations and overlap with other syndromes (Ellis-van Creveld syndrome [EVC]; 66 cases). The data include an incidence of consanguinity of 43.2% in RS and 45.3% in EVC. In RS, five out of eight sib pairs were discordant for at least one of the four cardinal phenotypic features of short stature (SS), fetal facies, mesomelic shortening and genital hypoplasia (GH). Two of these four sib pairs were also discordant for SS. In EVC, four out of fifteen sib pairs were discordant for congenital heart disease but concordant for the remaining diagnostic features of chondrodystrophy, ectodermal dysplasia and postaxial polydactyly. Assuming that the sib pairs have inherited the same disease causing mutation(s), the phenotypic discordance can be explained by the effect of modifier genes on specific clinical features in these syndromes, with the implication that recurrence risks may be variable for physical traits within malformation syndromes.

Delineation of a novel 5q35.3 microdeletion syndrome. *A.M. Rauch¹, M. Beese², H.G. Doerr², E. Mayatepek³, D. Wenzel², U. Trautmann¹.* 1) Institute of Human Genetics, Friedrich-Alexander University, Erlangen, Germany; 2) Department of Pediatrics, Friedrich-Alexander University, Erlangen, Germany; 3) Department of Pediatrics, University Heidelberg, Germany.

Recently 2.2 Mbp-microdeletions within chromosomal band 5q35 were identified as major cause of Sotos syndrome, an overgrowth syndrome with characteristic facial appearance, hypotonia and developmental delay (Kurotaki et al. 2002, Nat Genet 30:365-366). However, a patient with subtle cytogenetically visible deletion 5q35.3 in addition to overgrowth, hypotonia and mild developmental delay showed a short neck with nuchal edema, bell-shaped chest, and a very flat nasal bridge with epicanthus and large, retroverted ears (Stratton et al. 1994, Am J Med Genet 51:150-152). We observed a further patient with a very similar phenotype including short neck with nuchal edema, bell-shaped chest, a very flat nasal bridge with epicanthus and large, retroverted ears, but instead of overgrowth and advanced bone age presenting with shortness of stature and delayed ossification with huge anterior fontanel. Despite pronounced hypotonia and delayed motor development, speech development was within normal limits. While karyotyping after GTG banding showed normal results on a 850 bands level, subtelomeric analysis revealed a cryptic terminal 5q35.3 deletion. Further delineation of the deletion size with additional BAC clones showed a 3 Mbp-microdeletion distally adjacent to the Sotos syndrome deletion site. The phenotype of the patient described by Stratton et al. can now be explained as a compound phenotype of Sotos and the novel distal 5q35.3 microdeletion syndrome. As the Sotos syndrome microdeletion commonly occurs within the same boundaries, flanking low copy repeat regions are likely. Because the breakpoint of our patient is at the site of the assumed distally located low copy repeat, this novel microdeletion syndrome may occur more commonly. This syndrome will be of major impact also for prenatal diagnostics, as the nuchal edema in our patient was recognized in early pregnancy by increased fetal nuchal translucency.

Hoyeraal-Hreidarsson (HH) syndrome in three children. *Y. Sznajer¹, Cl. Baumann¹, F. Mechinaud³, A. David³, H. Journel⁴, D. Genevive¹, M. Raoul¹, M. Dumont², J-F. Segura², J-P. Cezard², T. Vulliamy⁵, I. Dokal⁵, A. Verloes¹.* 1) Clinical Genetics, Hôpital Robert Debré, Paris, France; 2) Department of Gastroenterology Hôpital Robert Debré, Paris, France; 3) Department of Clinical Genetics, Hôpital Mère Enfant, Nantes France; 4) Department of Clinical Genetics, Vannes, France; 5) Department of Haematology, Imperial College of Sciences, Technology and Medicine, Hammersmith Hospital, London, England.

Hoyeraal-Hreidarsson syndrome - a severe variant of X-linked dyskeratosis congenita - is characterized by Intra Uterine Growth Retardation (IUGR), cerebellar hypoplasia, microcephaly, immunodeficiency and aplastic anemia. We report on three patients with HH syndrome. Two brothers from unrelated parents presented IUGR. There was no family history. They developed failure to thrive with chronic diarrhea during the first year of life. Skin depigmentation, hair loss, mouth and tongue ulcerations and nail dystrophy were noted. They developed thrombopenia and pancytopenia. Patient 3, fourth child from a healthy mother, had severe IUGR. At the age of 7 months, he developed a first episode of mastoiditis and diarrhea. Aplastic anemia, thrombopenia and then pancytopenia were noted. In the first two patients, on bowel biopsies, epithelium was unusually dystrophic and atrophic; exsudative phenomena and microkystic glandular changes were found in all three. Brain Magnetic Resonance Imaging showed cerebellar atrophy in all three cases. They all died before the age of five. PCR Amplification followed by restriction enzyme digestion indicated a mutation (hemizygous) of DKC 1 gene (146 - C to T) in patient one and two as for their mother. In patient three, the analysis did not show any mutation. Our three patients developed HH syndrome. This was confirmed by mutation analysis in the first two cases and facilitated the genetic counselling. Not all cases of HH syndrome are identified by mutation analysis and this may account for possible genetic transmission heterogeneity.

Endocrine studies in subjects with Prader-Willi syndrome and simple obesity. *M.G. Butler¹, K. Kibiryeveva¹, D. Bittel¹, Z. Talebizadeh¹, T. Thompson².* 1) Childrens Mercy Hospital and University of Missouri Kansas City School of Medicine, Kansas City, MO; 2) University of Kansas Medical Center, Kansas City, KS.

Prader-Willi syndrome (PWS) is usually due to a 15q1-q13 deletion or maternal disomy 15 (UPD). Features in this syndrome can overlap with hypothyroidism or growth hormone deficiency. We analyzed thyroid and endocrine data in 48 PWS subjects (27 females, 21 males; 27 deletion, 21 UPD; mean age + SD = 22.9 + 8.5y) and 29 obese comparison subjects (20 females, 9 males; mean age + SD = 27.1 + 12.9y). No difference was seen in thyroid stimulating hormone levels between PWS or obese groups (2.2 + 1.3 vs 2.2 + 1.6 mcu/ml, respectively) or between PWS-deletion or PWS-UPD (2.4 + 1.5 vs 2.0 + 0.9 mcu/ml, respectively); however, significantly lower T3 levels were observed in the PWS group compared with obese (136 + 37 vs 158 + 32 ng/dl; $p = 0.02$, respectively). Free thyroxine levels were significantly lower in PWS compared with obese (1.12 + 0.21 vs 1.32 + 0.57 ng/dl; $p=0.04$, respectively) while total thyroxine levels were significantly lower but only in PWS females compared with obese females (7.86 + 2.14 vs 9.97 + 2.63ng/dl; $p=0.01$, respectively). Testosterone levels were lower in PWS males compared with obese males (1.27 + 1.02 vs 2.79 + 1.66ng/ml; $p = 0.01$, respectively) but no difference was found in the PWS-deletion or UPD. Estrogen levels were lower in PWS females than obese females (28.5 + 16.1 vs 74.1 + 55.9pg/ml; $p = 0.001$, respectively). Follicle stimulating hormone levels were similar in the PWS or obese groups but leutinizing hormone was lower in PWS compared with obese (1.9 + 1.8 vs 4.0 + 4.7miu/ml; $p = 0.01$, respectively) and a significant difference found in PWS females compared with obese females (1.5 + 1.3 vs 4.7 + 5.9miu/ml; $p = 0.01$, respectively). Growth hormone (GH) levels were low in both groups (PWS 0.74 + 0.16 vs obese 0.64 + 0.14ng/ml). Plasma IGF-I levels were significantly lower in PWS compared with obese (122 + 15 vs 174 + 21ng/ml; $p<0.05$, respectively) indicating GH deficiency [78% of PWS subjects had IGF-I levels < 10%ile for age compared with 43% of obese controls ($p<0.05$)]. GH is an important mediator for adipose tissue lipolysis and fat oxidation.

Multiple Sclerosis in an Individual with Maternal Uniparental Isodisomy for Chromosome 14. *V.R. Sutton¹, J.M. Greally², L.G. Shaffer¹*. 1) Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Departments of Medicine and Molecular Genetics, Albert Einstein College of Medicine, Bronx, NY.

Our 32 year old, previously-reported patient with maternal uniparental disomy for chromosome 14 (UPD 14) is the oldest known individual with this disorder. She recently has been diagnosed with multiple sclerosis (MS) based on MRI white matter changes and presence of oligoclonal bands in the cerebrospinal fluid. Both the T-cell receptor (TCR) alpha chain and TCR delta chain (nested within the alpha chain) are encoded by genes located on 14q11.2. Expression of TCRs occurs in a very specific order and determines which T-cells express alpha/beta TCRs and which express delta/gamma TCRs. We hypothesize that this region containing the alpha and delta TCRs may be subject to parent of origin effects and that maternal UPD 14 could alter the normal sequence of TCR rearrangement and expression. A scan of the DNA content across the region shows a trough of SINE content and locally high L1 content. This combination can indicate a paternally-expressed imprinted region. We hypothesize that over-expression of some relevant TCRs may have occurred at some critical time point, perhaps in the thymus, and this could then have resulted in abnormalities of tolerance mechanisms eventually leading to an autoimmune disease. We present clinical information, data on the SINE content of the region and a detailed analysis of the possible mechanisms whereby maternal UPD 14 could result in autoimmunity.

Pilot study to assess diabetic nephropathy in the HBDI collection of diabetic sib pairs. *J. Sowinski¹, K. Gogolin Ewens², C.A. Henry¹, R.S. Spielman².* 1) NDRI/HBDI, Philadelphia PA; 2) Dept of Genetics, Univ of Pennsylvania School of Medicine, Philadelphia, PA.

We have carried out a pilot study to re-contact diabetic offspring in 45 families that are part of the HBDI collection of multiplex families identified on questionnaires as having diabetic nephropathy (DN). This collection is especially valuable since DNA has already been collected and banked for all parents and diabetic siblings. In addition, the long duration of diabetes (mean=24.6 years) makes most offspring informative for DN or absence of DN despite long-term diabetes. Updated medical information, including the presence of end stage renal disease (ESRD) and information on relevant medications (ACE inhibitors and AGTR1 blockers), was obtained from 93 (82%) of 113 diabetic offspring in the 45 families. DN status was determined by measuring the albumin/creatinine ratio (ACR) in two or three spot urine samples collected at least 6 weeks apart. Phenotypes were assigned as DN (ESRD or overt proteinuria with ACR >300 mg/mg), or unaffected for DN (normoalbuminuria with ACR <30 mg/mg, duration of diabetes >15 years and not taking any relevant medications). Diabetic individuals with duration <15 years, ACR 30-300ug/mg or ACR <30 mg/mg but taking relevant medications could not be classified into either group.

Of the 93 individuals studied, 34 (37%) were affected with DN and 18 (19%) unaffected with DN; 27 (29%) could not be classified into either group. Fourteen individuals (15%) are still of unknown status. Thus we are currently able to assign a nephropathy phenotype (affected or unaffected) to 56% of the individuals in this study, and expect that number to reach 65-70% when the project is completed. Based on the results of this pilot study, we are now beginning to re-contact 390 families in the HBDI collection for whom contact information is available. We expect that we will be able to assign informative nephropathy phenotypes to at least 200-250 diabetic families, which will significantly increase the number of trios and multiplex families available for studies of the genetics of diabetic kidney complications.

HENNEKAM SYNDROME PRESENTING AS NONIMMUNE HYDROPS FETALIS, CONGENITAL CHYLOTHORAX, AND CONGENITAL PULMONARY LYMPHANGIECTASIA. *C. Bellini, M. Mazzella, C. Arioni, G. Serra.* Servizio Patologia Neonatale, Universita Genova, Istituto Gaslini, Genova, Italia.

Case Report. During pregnancy polyhydramnios had become evident at 30 weeks of gestation. At birth (33 weeks) respiratory problems due to hydrops fetalis urged immediate intubation, artificial ventilation, and surgical draining; a chylothorax was diagnosed. Physical examination showed a flat face, epicanthal folds, a broad, depressed nasal bridge, a bulbous nasal tip. Generalized lymphedema was evident. Laboratory examinations showed hypoproteinemia and hypogammaglobulinemia. Repeated examinations of faecal excretion of alpha-1-antitrypsin showed elevated values (550 to 650/gr wet), suggesting intestinal lymphangiectasia. Chromosome studies (46, XX), TORCH titer, plasmatic 7-dehydrocholesterol level, renal and cardiac ultrasonography, gave normal results. High Resolution Computed Tomography at 45 days of age confirmed pulmonary lymphangiectasia. At the age of 55 days radionuclide lymphoscintigraphy of hands and feet showed abnormal drainage of the lower limb and the thoracic duct. Initial dermal back-flow was evident in the distal hand and foot. Back-flow within the thoracic duct was evident. In 1989, Hennekam and co-workers reported a new autosomal recessive entity (OMIM 235510) characterized by lymphedema of limbs, genitalia and face, secondary facial dysmorphic features, intestinal lymphangiectasia, and varying degrees of mental retardation. Since the first report 24 patients have been described. Here we report on a newborn girl with this entity who presented with nonimmune hydrops fetalis, and was found to have a congenital chylothorax and pulmonary lymphangiectasia. Generalized maldevelopment of the lymphatic system is the main characteristic of the syndrome, preferentially affecting intestines, limbs, genitalia, but it can also affect the pleura, pericardium, thyroid gland, kidneys. Pleural lymph vessel anomalies have been reported in 7/21 cases. An important point of this work is the original demonstration obtained by lymphoscintigraphic study of the direct involvement of the thoracic duct in the pathogenesis of nonimmune hydrops fetalis, chylothorax, and pulmonary lymphangiectasia.

Altered lung function in Fabry's Disease (FD). *S. Magage, A. Linhart, J.C. Lubanda, Z. Susa, J. Bultas, D. Karetova, M. Elleder, J. Zeman.* Cardiology, Charles University, Prague, Czech Republic.

Background: Fabry disease is an X-linked hereditary metabolic disorder caused by deficient levels of the lysosomal enzyme α -galactosidase A leading to intracellular accumulation of neutral glycosphingolipids within many organ systems. Bronchopulmonary involvement has been reported but pulmonary function findings were never compared to a control population.

Objectives: To compare spirometric values obtained in patients with confirmed Fabry disease with healthy volunteers.

Materials and methods: Spirometry was performed in 50 Fabry disease patients (23 males and 27 females), and in 50 gender, age, allergic reactions and smoking habit matched healthy volunteers. A set of spirometric variables (FVC, FEV₁, and FEF₂₅₋₇₅) was obtained. FEV₁ >80% and FEF₂₅₋₇₅ <70% defined small airway obstruction, FEV₁ of 60-79% = mild obstruction, FEV₁ 50-59% = moderate obstruction, and FEV₁ <50% = severe bronchial obstruction.

Results: All mean spirometric variables (% predicted) were lower in affected males than in females. FVC (% predicted) was reduced 15.4% ($97.7 \pm 18.7\%$ in Fabry patients vs $110.3 \pm 13.6\%$ in normal controls; $p < 0.001$). FEV₁ (% predicted) was reduced 20.6% ($81.5 \pm 19.8\%$ in Fabry patients vs $102.6 \pm 11.6\%$ in normal controls, $p < 0.0001$). FEF₂₅₋₇₅ (% predicted) was reduced 43.1% ($48.4 \pm 19.7\%$ in Fabry patients vs $85.1 \pm 18.7\%$ in normal controls; $p < 0.0001$). Evidence of bronchial obstruction was present in 40 Fabry patients (80%) of whom 25% demonstrated small airway disease; 5 had mild, 3 moderate and 2 severe obstruction. Small airway obstruction alone was detected in 12 control individuals ($p < 0.0001$).

Conclusions: Fabry patients demonstrate significantly lower spirometric parameters than normal controls, suggesting a reduction in ventilatory capacity and the presence of bronchial obstruction. Hemizygous males are significantly more severely affected than heterozygous females.

Phenotype description of Van der Woude Syndrome (VWS) unlinked to chromosome 1 containing IRF6. H. Okur¹, I. Vargel², S. Kondo³, B.C. Schutte³, F. Ozgur², S. Balci⁴, S. Ozusta⁵, I. Cekirge⁶, B. Girgin¹, Y. Erk², J.C Murray³, A.N Akarsu¹. 1) Gene Mapping Laboratory, Ped.Res.Cent. Hacettepe Univ; 2) Dept. of Plastic and Reconstructive Surgery, Hacettepe Univ; 3) Dept. of Pediatrics, University of IOWA, IA, USA; 4) Departments of Clinical Genetics; 5) Child Psychiatry; 6) Radiology, Hacettepe University, Sıhhiye, 06100, Ankara, Turkey.

VWS is a malformation manifesting cleft lip(CL)and/or palate (CP),lower lip pits (P) and hypodontia. Significant abnormalities in brain structure and function were also described. Associations such as poplyteal pterygium, ankyleblepharon filiforme and syngnathia were commonly reported. First VWS locus has been mapped to chromosome 1q32-q41 with remarkable genetic homogeneity in all populations. A second locus was reported to 1p34 using a large Finnish pedigree. The Finnish pedigree linked to 1p34 closely resembled non-syndromic forms of CL/P and suggested that sub-phenotypes may exist in VWS. We have previously presented two distinct Turkish families with “typical” as well as “complicated” forms of VWS and reported no linkage to chromosome 1q32-q41 in the complicated one (*AJHG*; 2001;69: 289 Abstract 617). CP(80%);P(60%) and CL(46%)were the clinical findings of typical VWS family linked to 1q32 region. Major striking observation in the complicated family is that affected members segregate with CL(100%) and P(30%) without CP in at least five generation. Additionally, ankyleblepharon filiforme and syngnathia was observed in one affected case. Normal intelligence and brain structure were detected in affected members. Genetic linkage analysis excluded both VWS loci using 16 DNA markers. Recently, mutations in Interferon Regulatory Factor 6 (IRF6) gene were identified as a cause of Van der Woude and Popliteal Pterygium syndromes. No mutation has been detected in the coding region of IRF6 gene for “complicated” family whereas a missense mutation in exon 4 including the DNA binding domain(nt:A332G; aa:Y111C) was detected in the family with typical findings. Thus, the family presenting CL (without CP), pit, ankyleblepharon filiforme, normal brain structure and function could be another example of sub-phenotype of VWS. *nakarsu@hacettepe.edu.tr*.

Small terminal deletion of chromosome 9q34: a novel recognizable phenotype? V. Cormier-Daire, F. Molinari, M. Rio, M.C. de Blois, S. Romana, M. Vekemans, A. Munnich, L. Colleaux. INSERM U393 and Department of Genetics, Hopital Necker, Paris, France.

Cryptic subtelomeric chromosome anomalies have been recognized as a significant cause of idiopathic mental retardation. Some microscopic deletions such as 4p-, 5p-, 9p- or 13q- are associated with distinctive clinical features and can, therefore, be ascertained through specific phenotypes. However, the phenotype of the various subtelomeric deletions is largely unknown.

Here we report the clinical and molecular findings in two unrelated mentally retarded boys with a small *de novo* submicroscopic deletion of chromosome 9q34 region. Both patients shared a clinical pattern of severe mental retardation, abnormal genitalia (hypospadias and cryptorchidism) and dysmorphic features characterized by flat face, high forehead, synophrism, anteverted nostrils, long philtrum, thin upper lip, protruding tongue and short extremities. They also developed progressive obesity with food seeking behaviour (with weight $> +6$ SD in the oldest one at 9 years of age), hyperactivity, sleep disturbance and absence of speech. The size of the telomeric deletion is about 3 Mb. These findings are discussed in light of the detailed molecular analysis of the rearrangement. Although several of these clinical manifestations can be observed in other chromosomal disorders, their combination could be a distinctive feature suggesting that this association defines a novel clinical entity.

Cerebro-Oculo-Facio-Lymphatic syndrome (COFL): A new MR/MCA syndrome with proposed cardinal diagnostic features. *J.M. Milunsky¹, D.M. Capin²*. 1) Ctr Human Genetics and Dept. Pediatrics, Boston Univ Sch Medicine, Boston, MA; 2) Pediatric Neurology, Cambridge Hospital, Cambridge, MA.

Fryns and Aftimos [2000] described a new MR/MCA syndrome with distinct facial appearance and general habitus, broad and webbed neck, hypoplastic inverted nipples, epilepsy, and pachygyria especially of the frontal lobes. We evaluated a 12 year old male with profound mental retardation, seizures, and MCA including lissencephaly, facial dysmorphism, and lymphatic abnormalities, who had clinical features remarkably similar to the initial two cases reported. Our Brazilian proband was born at 28 weeks gestation to a non-consanguineous couple with a noncontributory family history. Tonic and myoclonic seizures were first noted at 8 months and seizures have continued daily despite multiple anti-epileptic medications. He has never walked and has single words only. At 12 years, his weight was 10th centile, length 5th centile and OFC less than 3rd centile. General habitus included wasted muscle mass, knee and elbow contractures, and bilateral equinovarus deformity. Craniofacies is notable for bitemporal narrowing, trigonocephaly, thick hair, facial edema, bilateral ptosis, hypertelorism, epicanthal folds, broad nasal bridge, bulbous nasal tip, protuberant posteriorly rotated ears, a thin upper vermilion border, everted lower lip, long flat philtrum, macrostomia with prominent upper central incisors, and micrognathia. He has a high arched palate, a broad, short, webbed neck with a low posterior hairline, pectus excavatum, kyphosis and wide inverted hypoplastic nipples. Loose skin on his hands, which appeared short and broad along with tapered fingers are noted. He has a shallow scrotum and small testes. Hypertonia, spastic paraparesis, rotary nystagmus, and strabismus are present. Head MRI demonstrated lissencephalic cortical dysplasia with anterior pachygyria. High-resolution chromosomes, subtelomeric FISH, Miller-Dieker FISH, and metabolic testing including cholesterol and 7-dehydrocholesterol did not reveal an underlying diagnosis. We propose a name (COFL syndrome) for this disorder, suggest cardinal diagnostic features, and discuss several possible overlapping syndromic diagnoses.

Mutation Analysis and Phenotypic Description of Three Severely affected Smith-Lemli-Opitz Syndrome Patients.
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Smith-Lemli-Opitz Syndrome (SLOS) is an autosomal recessive malformation syndrome caused by mutations in the gene encoding the sterol delta-7 reductase. This protein catalyzes the reduction of 7-dehydrocholesterol to cholesterol in the last step of cholesterol biosynthesis. To date over 70 different mutations have been reported. Failure to ascertain severe cases, due to prenatal and perinatal death, is thought to skew reported allele frequencies. Here, we report the clinical and molecular data of three severely affected SLOS patients. The first patient was a 46,XY infant who died at 2 months of age. This infant had alobar holoprosencephaly, cleft lip, cleft palate, polydactyly, syndactyly and ambiguous genitalia. The ratio of 7-dehydrocholesterol/cholesterol was 73%. Both parents were heterozygous for the IVS8-1G>C mutation. The second patient was a 46,XY infant with ambiguous genitalia, who died at 12 hours of age. Congenital heart malformation consisted of hypoplastic left ventricle, dysplastic mitral valve, and patent foramen ovale. The biochemical studies revealed markedly increased 7-dehydrocholesterol level of 39 ug/ml and reduced level of cholesterol of 8.1 mg/dl. The DHCR7 genotype in this case was IVS8-1G>C/T319A. The third patient was a 46,XY infant with micrognathia, cleft palate, cataracts, postaxial polydactyly and ectrodactyly. 7-DHC was elevated at 80 ug/ml and cholesterol was 6mg/dl. Genotype was W151X/T154R. Genotypes identified in these three severely affected SLOS infants were W151X/T154R, IVS8-1G>C/T319A and IVS8-1G>C/IVS8-1G>C. PCR/RFLP assays were developed to confirm the T154R and T319A mutations. Genotyping of severely affected infants is important to avoid skewing of allele frequencies determined from less severely affected, SLOS populations.

Normal cognition and behavior in a Smith-Lemli-Opitz syndrome patient who presented with Hirschsprung's. C. Mueller¹, S. Patel², M. Irons³, K. Antshel³, G.S. Tint⁴, C. Bay⁵. 1) Magee Women's Hospital, Pittsburgh, PA; 2) Medical University of South Carolina, Charleston, SC; 3) Children's Hospital, Boston, MA; 4) VA Medical Center, East Orange, NJ; 5) Children's Hospital, Pittsburgh, PA.

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive disorder of cholesterol metabolism. It is caused by mutations in the gene for 7-dehydrocholesterol-D7-reductase (DHCR7), which catalyzes the final step in cholesterol biosynthesis, and results in cholesterol deficiency. We present the case of a 3.5 year old female child with normal cognition and behavior in whom molecular studies have identified two missense mutations in DHCR7: V326L and F248L. She was born at term following an uncomplicated pregnancy and delivery, and presented 12 days of age with poor feeding, abdominal distention, and jaundice. Colonic biopsy was consistent with Hirschsprung disease. On physical examination she had mild ptosis, 2,3 syndactyly borderline on one foot and 1mm above significance on the other, micrognathia, and a short, upturned nose. Her 7-dehydrocholesterol level was markedly elevated at 87mg/ml (normal 0.10+/-0.05) and her cholesterol level was normal at 61 mg/dl (normal 50-80mg/dl). Karyotype was 46,XX. Breast milk feeding was initiated and continued for 18 months. Cholesterol supplementation was implemented at 100mg/kg/day at 3 months, which has resulted in increased cholesterol levels and much lower dehydrocholesterol levels. Neuropsychological testing shows her functioning in the low average range, between the 14-18th centile when compared to same-age peers. This is markedly higher than most children with SLOS. She has no behavioral problems. MRI and MRS testing of the brain revealed no structural abnormalities. This is in contrast to a recently reported case by Prasad (AJMG 2002) with a mild phenotype, behavioral problems, and abnormal MRI, who is compound heterozygote for both a null and missense mutation. A higher residual enzyme activity could be the explanation for the milder clinical phenotype and normal cognition and behavior of our patient. Feeding abnormalities may be the most consistent feature of SLOS. Hirschsprung disease could be an indication for testing.

Carrier Frequency of the Smith-Lemli-Opitz IVS8-1G>C mutation of the DHCR7 gene in African Americans.

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Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive multiple congenital anomaly/mental retardation syndrome due to a deficiency of 3 β -hydroxysterol D⁷-reductase (DHCR7) activity. DHCR7 catalyzes the reduction of 7-dehydrocholesterol (7DHC) to form cholesterol via the Kandutsch-Russell pathway. SLOS is thought to be most prevalent in individuals of northern and central European descent with the incidence estimated to be between 1/10,000 and 1/60,000. The most common SLOS mutation is IVS8-1G>C, which accounts for approximately one third of reported SLOS alleles. The carrier frequency of the IVS8-1G>C mutation has been reported to be on the order of 1% in North America. SLOS is thought to be rare in individuals of African descent, and the description of only one African American patient has been reported. To define the carrier frequency of the IVS8-1G>C SLOS mutation in the African American population, 1378 anonymous African American newborn whole blood spot samples were screened for the mutant allele using an amplification refractory mutation system (ARMS) assay. Ten of the 1378 samples contained the IVS8-1G>C allele, and all ten positives were determined to be heterozygous for the mutation. This indicates a carrier frequency of 0.73% with a 95% confidence interval of 0.35-1.33%. This would predict an SLOS incidence of 1/75,000 in African Americans. Consistent with previous work showing that the IVS8-1G>C mutation exists on haplotype A, at least eight of the IVS8-1G>C carriers were found to be haplotype A on one or more alleles. Haplotype analysis was ambiguous in two samples, but haplotype A was indicated as a possibility in both cases. These results suggest that SLOS may more prevalent in African Americans than previously thought.

Novel IGHMBP2 mutations in Spinal Muscular Atrophy with Respiratory Distress (SMARD1). *L. Viollet, M. Zarhrate, P. Burlet, S. Lefebvre, A. Munnich.* INSERM U393 and Department of Genetics, Hopital NECKER, Paris, France.

Spinal Muscular Atrophy with Respiratory Distress -also known as diaphragmatic spinal muscular atrophy or Distal Hereditary Motor Neuronopathy type VI- is a rare autosomal recessive neuromuscular disorder characterized by progressive anterior horn cell degeneration, leading to neurogenic muscular atrophy with early lifethreatening respiratory insufficiency. In contrast to classical autosomal recessive spinal muscular atrophy (SMA), distal muscles are severely affected in SMARD and diaphragmatic paralysis is the most prominent presenting symptom. Clinical and genetic heterogeneity of SMARD has been previously shown and one form, characterized by the onset of respiratory distress during the first weeks of life (SMARD1), has been ascribed to chromosome 11q13-q21 (Grohmann et al, 1999). Recently, seven mutations have been reported in the immunoglobulin m-binding protein 2 gene (IGHMBP2), in six unrelated SMARD1 families (Grohmann et al, 2001). This gene, also mapped to 11q13, is homologous to the mouse IGHMBP2 gene, which accounts for spinal muscular atrophy in the neuromuscular degeneration mouse (nmd). The cellular function of IGHMBP2 is still unknown. We report here on compound heterozygosity for IGHMBP2 mutations in a Caucasian male patient, presenting a typical SMARD1 phenotype with onset at the age of 4 weeks. These mutations were detected by DHPLC analysis of the IGHMBP2 sequence and confirmed by direct double strand DNA sequencing. One mutation was a T752C substitution which changed a leucine into a proline (L251P) in a conserved residue of the protein. This substitution resulted in the creation of an HpaII cleavage site. The other mutation is a T1730C substitution, changing a leucine into a proline (L577P) in one of the putative helicase domains of IGHMBP2. For this second mutation, SSCP analysis of the mutated DNA fragment revealed a specific band shift. These two variants were not identified by restriction digestion with HpaII and SSCP analysis in 200 chromosomes of unrelated healthy subjects. The identification of novel IGHMBP2 variants will help to diagnosing SMARD1 and may contribute to the functional characterization of IGHMBP2 gene product.

The Natural History of Bardet-Biedl Syndrome (BBS) by Genotype: Incident Events in a Longitudinal Study. S.

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BBS (OMIM:209900) is a genetically and clinically heterogeneous disorder characterised by retinal dystrophy, obesity, renal abnormalities, dystrophic extremities and hypogenitalism. Forty-six patients with Bardet-Biedl syndrome (BBS) from 26 families in Newfoundland were entered into a longitudinal study (started in 1985). Medical charts were reviewed for all cases, and 25/46 were examined in 2001. Patients from 5 families were linked to the BBS1 locus on 11q13, one to 16q21 (BBS2), 5 patients from 1 pedigree linked to 3p12 (BBS3), and 5 patients from another family linked to 2q31 (BBS5). In a further 12 patients from 6 families, a mutation was identified in the *MKKS* gene associated with BBS6. The genotype was unknown in the other 17/46 (37%) patients from 12 families (2 patients from 1 family were excluded from all loci). The Kaplan-Meier method was used to analyse age of onset of clinical endpoints, including blindness, chronic renal failure, hypertension, diabetes mellitus and death. The visual loss was severe and early onset for all genotypes, with a median age of 18 years to register blind. Early-onset hypertension was common, occurring in 69% (median age of onset 34 years). Forty-five percent of patients had diabetes mellitus (median age of onset 45 years). Chronic renal failure occurred in 37%, with a median age of onset of 60 years; 4 developed end stage renal disease. These outcomes were similar for all genotypes. Twelve patients died, the median age of death was 62 years. These data suggest that the natural history of BBS does not differ by genotype, and the BBS genes are involved in a common embryogenic pathway.

Puberty and adolescence in Cornelia de Lange syndrome: a survey of 67 patients. *A.D. Kline¹, L. Audette²*. 1) Harvey Inst Human Gen, Greater Baltimore Medical Ctr, Baltimore, MD; 2) Cornelia de Lange Syndrome Foundation, Avon, CT.

Few publications address adolescent and young adulthood issues in genetic syndromes. CdLS patients originally evaluated as infants or young children are now entering adulthood. To further understand pubertal changes, 67 puberty questionnaires distributed to member families of the CdLS Foundation have been evaluated to date. Two additional surveys have been received on female children with precocious puberty. The average age of puberty onset was 13 years in females and 14 years in males. Among 43 females with CdLS, the following data regarding pubertal changes were reported: 37% growth spurt in height, 53% growth spurt in weight, 93% pubic hair, 88% breast development, 19% axillary hair, 70% menses, and 7% lack of menarche. Two individuals were found to have a bicornuate uterus with a duplicated cervical os. Premenstrual syndrome is common and menstrual irregularity is noted. Control of menses include hormone replacement therapy, hysterectomy and gynecologic procedures. 24 responses regarding pubertal changes on males with CdLS indicated: 50% growth spurt in height, 33% growth spurt in weight, 92% pubic hair, 29% axillary hair, 67% facial hair and 29% increased size of genitalia. 43% of all patients reported worsening gastroesophageal reflux during puberty. Other medical problems include scoliosis, nasal polyps, seizures and psychiatric disorders. 64% reported problems with circulation, including cold, painful extremities with changes in color. Behavioral issues are extremely common and worsen with the onset of puberty, including aggression, self-injury, obsessive-compulsive tendencies, oppositional behaviors and moodiness. Sleep patterns change, and masturbation is mentioned. Management of behavioral issues provides the most difficult challenge to the caregivers. Only by following patients with syndromes through adolescence and into adulthood, will geneticists be able to address peripubertal and adult issues and advise both the families and the primary care providers about implications for health care, changes in family dynamics, and prognosis. In addition, this type of information will be helpful for genotype-phenotype correlations.

The phenotype of normal tension glaucoma patients with and without OPA1 polymorphisms. *T. Aung*^{1,2,3}, *K. Okada*^{3,4}, *D. Poinosawmy*³, *L. Membrey*³, *G. Brice*^{3,5}, *A. Child*^{3,5}, *S. Bhattacharya*¹, *O. Lehmann*^{1,3}, *D. Garway-Heath*³, *R. Hitchings*³. 1) Molecular Genetics, Institute of Ophthalmology, London, London, United Kingdom; 2) Singapore National Eye Centre, Singapore; 3) Moorfields Eye Hospital, London, UK; 4) Department of Ophthalmology, Hiroshima University Faculty of Medicine, Hiroshima, Japan; 5) St Georges Hospital Med School, London, UK.

Purpose. Polymorphisms in *OPA1*, the gene responsible for autosomal dominant optic atrophy, were recently found to be strongly associated with normal tension glaucoma (NTG). The aim of this study was to determine whether *OPA1* polymorphisms affect the phenotype of NTG patients. **Methods.** A retrospective analysis was performed of 108 well-characterized NTG patients who had been genotyped for *OPA1* variations, and had previously undergone automated perimetry and Heidelberg retina tomography (HRT). Twenty-five NTG patients had the at-risk *OPA1* genotype (IVS 8 +4 C/T; +32 T/C) and 83 NTG patients did not. Differences between groups were sought in a wide range of structural, psychophysical and demographic factors. For a subgroup of patients with at least 5 years of follow-up and 10 visual field tests, pointwise linear regression analysis (PROGRESSOR for Windows software) was applied to the visual field series. **Results.** There was no significant difference in the 2 groups with respect to sex, age at diagnosis, family history of glaucoma, history of ischaemic risk factors and vasospasm or laterality of glaucoma. The comparison of IOP, CD ratio and visual field global indices, MD and CPSD in the 2 groups showed no significant difference. There were no differences in the mean values for any of the HRT parameters analyzed. For the subgroup of patients with at least 5 years of follow-up, there was also no significant difference in the number of patients with progressing locations, the mean number of progressing locations per subject, the mean slope of the progressing locations or the mean slope for whole visual field. **Conclusions.** The absence of phenotypic differences in NTG patients with and without the *OPA1* polymorphisms IVS 8 +4 C/T; +32 T/C suggest that these *OPA1* polymorphisms do not underlie any major phenotypic diversity in these patients.

Severe Myoclonus-Dystonia syndrome associated with a novel epsilon-sarcoglycan gene truncating mutation. *L.*

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Myoclonic-dystonia (MDS) is an autosomal dominant disorder characterized by myoclonic and dystonic muscle contractions, associated to psychiatric manifestations. MDS is usually considered as a benign disease. In most of the families, MDS is linked to chromosome 7q21 and mutations within the epsilon-sarcoglycan (*SGCE*) gene have been recently described. We report the detailed phenotype, including neurological, neuropsychological and psychiatric assessment, of a large French MDS family. The phenotype in this family was severe and heterogeneous, including myoclonus with important functional impact and several psychiatric features, characterized by depression, obsessive-compulsive disorders and anxiety. This phenotype was shown to be associated to a novel truncating mutation located within exon 4 of *SGCE*. Asymptomatic mutation carriers carried the mutation on their maternal chromosome in agreement with previous segregation analysis of MDS families linked to the *SGCE* locus, which suggested that the penetrance of the mutation is highly dependent on the parental origin of the disease allele. This report demonstrates that *SGCE* mutation can result into a severe phenotype not compatible with normal life.

DYSMORPHOLOGY ASSOCIATED WITH IN-UTERO COCAINE EXPOSURE. *N.H. Robin*^{1,2}, *A.A. Alt*², *B. Salbert*³, *L. Ellison*², *S. Minnes*², *L. Singer*². 1) Center for Human Genetics, Dept. Genetics; 2) Dept. Pediatrics, Univ Hosp Cleveland, CWRU Sch Med, Clev, OH; 3) Clinical Genetics, MetroHealth Med Ctr., Cleveland, OH.

The effects of prenatal cocaine exposure (CE) have been debated for some time. While some studies have not shown any adverse effects, others have demonstrated a variety of neurodevelopmental sequelae. Similarly, the question of CE causing physical anomalies is also unresolved. The possibility that it causes a distinctive phenotype was suggested, but a blinded study of cocaine exposed newborns failed to confirm the existence of a cocaine syndrome. Methods: To further explore the relationship between CE and dysmorphology, we have evaluated 208 6-year-old children, 114 CE, and 94 age matched controls non CE. While the groups were divided by CE, exposure to nicotine, alcohol, and marijuana was also identified and used for phenotype correlations. Each child underwent a complete dysmorphology examination, with 10%; having a repeat exam to maximize reliability. The exam included a series of anthropomorphic measurements (e.g. head circumference, eye spacing, arm span), and a dysmorphologic examination to assess the presence or absence of 130 findings. Data was analyzed by SAS. Results: In preliminary analysis, few findings correlated with CE, alcohol, marijuana, or nicotine. CE correlated with palpebral fissure (PF) abnormalities: 11%; (13/114) CE vs. 3%; (3/94) NCE, $p=0.027$. However, there was no consistency among the PF anomaly - both long and short were noted. Alcohol exposure correlated with a higher rate of brachydactyly: 32%; (44/136) alcohol exposed vs. 18%; (13/72) non-exposed, $p=0.028$. Philtrum score negatively correlated with both nicotine and marijuana exposure, $p=0.0029$. Conclusions: We were unable to document a higher rate of dysmorphic findings among CE children in this cohort. While some abnormalities were noted, they did not occur in a consistent or recognizable pattern. This suggests that prenatal CE does not cause a distinct dysmorphic syndrome, nor a higher rate of minor anomalies. We agree with Little et al. [Teratol 54:145, 1999] that, if a congenital cocaine syndrome exists, it occurs at a low rate among CE children.

Oxycontin exposed fetuses and possible recurring facial phenotype. *B.D. Hall.* Dept Pediatrics/Genetics, Univ Kentucky Col Med, Lexington, KY.

Oxycontin is an opioid analgesic, which has a pregnancy classification as a Category B drug, with no fetal malformations in rats or rabbits of po exposed mothers. No human studies exist and no literature reports, anecdotal or otherwise, have dealt with the human experience. Kentucky was one of the first states to experience an epidemic of Oxycontin abuse with its increased rate of addiction and death. Most abuse results from breaking up the tablets and snorting, swallowing, or injecting the powder.

Four consecutive children ages 7 weeks to 10 months, whose mothers abused Oxycontin for all (N3) or part (N1) of their pregnancies, were referred for evaluation of dysmorphic features. Three of the children had normal pre-/postnatal growth and all 4 were within normal limits for psychomotor development. Three infants had neonatal withdrawal symptoms with one still showing hypertonia and tremors at 4 months. All 4 children had micrognathia and a small mouth while 2 had upslanted eyes; and each one had either a cleft palate, blunt upturned nose, simple philtrum, high forehead or overlapping toes.

The facial phenotype in these 4 Oxycontin exposed children is similar. If Oxycontin has teratogenic effects the resulting phenotype may be an important diagnostic clue. Experience with previous opioids such as morphine does not indicate an increased teratogenic risk. Nevertheless, further analysis of the offspring of mothers abusing Oxycontin is indicated.

Valproate embryopathy: Clinical and cognitive profile in 5 siblings. *E.K. Schorry, S.G. Oppenheimer, H.M. Saal.*
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Valproate embryopathy is a well recognized syndrome caused by prenatal exposure to the anticonvulsant valproic acid (Depakote). We report 5 half-siblings with the same mother (4 different fathers) who all have valproate embryopathy. Valproic acid was the sole anticonvulsant in all 5 pregnancies, with doses ranging from 500 mg-2000 mg per day. All children were examined by a clinical geneticist and had extensive developmental testing. Mean birth weight at term was 2950 gms (range 2400-3400 gms). Common features in the 5 children included: flat, broad nasal bridge (5/5), hypoplastic midface (4/5), apparent hypertelorism/telocanthus (3/5), smooth philtrum with thin upper lip (5/5), long thin tapering fingers (4/5), hypoplastic 5th toenails (2/5), and irregularly placed toes (2/5). Less frequent features were cleft palate (1/5), duplication cyst of small intestine (1/5), and hemangioma (1/5). None had neural tube defect. Cognitive testing of the 3 children above 4 years of age showed cognitive ability in the low normal or borderline range (mean IQ=83; range 75-86), with significantly lower scores in adaptive behavior and motor skills. Study of this family offers insight into the high risk of valproate embryopathy in exposed pregnancies, and affords a unique opportunity to study the variability of expression and cognitive profile of the syndrome within one family.

Endostatin measurement as preliminary screening test for the diagnosis of Knobloch syndrome. *O.T. Suzuki*¹, *F. Kok*², *V.M. Der Kaloustian*³, *B. Olsen*⁴, *M.R. Passos-Bueno*¹. 1) CEGH-USP, São Paulo, SP, Brazil; 2) FMUSP, São Paulo, SP, Brazil; 3) McGill University, Canada; 4) Harvard University, USA.

Knobloch syndrome (KS) is a rare autosomal recessive disorder characterized by high myopia, vitreoretinal degeneration with retinal detachment, macular abnormalities and occipital encephalocele. It is caused by mutations in the *COL18A1* gene, which encodes the extracellular matrix protein collagen XVIII (Sertié *et al.*, 2000, HMG, 9:2051). This gene includes 43 exons and its two distinct promoters regulate the transcription of 3 mRNA variants. Endostatin is a 20 kDa fragment produced by proteolytic cleavage of collagen XVIII and is common to all variants. This fragment originally characterized as a potent inhibitor of angiogenesis, also plays a role in apoptosis and endothelial cell proliferation. We measured endostatin plasma levels through an ELISA assay in 19 individuals (9 KS patients and 10 healthy relatives) from 5 unrelated families. Six patients bear mutations that affect only the short collagen XVIII variant (IVS1-2A®T), one patient bears mutations that affect all known variants (c2105delC / c3514-3515delCT) and 2 other patients did not reveal any mutations after screening the entire coding region of the gene. All the mutations found result in a truncated protein. Patients and healthy carriers for the mutation IVS1-2A®T present levels (from 17.9 to 32.7 ng/ml) comparable to the average value found in control individuals (20.3 ± 11.5 ng/ml). These findings support the hypothesis that circulating endostatin levels are most likely derived from the medium and long isoforms as these patients have deficiency of only the short form. Normal endostatin levels were also found in patients without detectable mutations, suggesting genetic heterogeneity or non-detectable mutations affecting only one variant. Low level (8.5ng/ml) was observed only in the patient with null alleles that affect all variants. This was unexpected, as lack of endostatin was observed in mice with *Coll18a1* null alleles (Fukai *et al.*, 2002, EMBO J, 21:1535). These results demonstrate that endostatin measurement may be used to help the KS diagnostic and the mutation screening. FAPESP, PRONEX, CNPq, HHMI.

The fragile X premutation and autistic spectrum disorders. *R.J. Hagerman¹, B.L. Goodlin-Jones¹, S. Spence², L. Albrecht¹, S. Bacalman¹, F. Tassone³, L.W. Gane¹, S.W. Harris¹, P.J. Hagerman³.* 1) M.I.N.D. Institute, UC Davis Medical Center, Sacramento, CA; 2) Autism Genetic Research Exchange and Department of Pediatrics, UCLA, Los Angeles, CA; 3) Department of Biological Chemistry, University of California, Davis, CA.

This paper reports on eight subjects, ages 3 years to 33 years, with the fragile X premutation and a diagnosis of Autistic Spectrum Disorder. In these 8 subjects the CGG repeats ranged from 56 to 180 while the FMRP levels were in the normal or mildly deficient range. All subjects exhibited features of fragile X syndrome, including cognitive and language problems, avoidance of eye contact, and some hand stereotypies. All subjects were assessed with standardized measures for autism (e.g. ADOS, ADI-R) as part of the clinical protocol. Five subjects made criterion for autism both clinically and with the standardized measures while three made criterion for autistic spectrum disorder (including Asperger and PDD-NOS). The subjects had autistic features including impairment in social reciprocity, repetitive behaviors, and impoverished social overtures which were observed in addition to the features of fragile X syndrome. Information will be presented about a range of mRNA elevations in these patients. One hypothesis may be that this association of autism and the fragile X premutation is related to the FMRP gene dysfunction, specifically a translation defect that begins in the premutation range. In addition, a second gene affect may be additive to the premutation to predispose patients to an autism spectrum disorder.

Fragile X and autism diagnosis by two standard methodologies. *S.W. Harris, B.L. Goodlin-Jones, S. Bacalman, T. Jardini, S. Rao, R.J. Hagerman.* M.I.N.D. Institute, UC Davis Medical Center, Sacramento, CA.

Autism is often associated with fragile X syndrome (FXS) and previous reports have shown that 15% to 33% of males with FXS have autism. The prevalence rates have varied depending on the measures used to document autism. Only one published study has utilized the ADOS and ADI-R that are considered the gold standards for the diagnosis of autism. That study reported that 33% of preschool children with FXS had autism. We will address the assessment of autism in a consecutive sample of 40 children with fragile X syndrome seen for comprehensive neurodevelopmental assessments. The mean age was 7 years and there were 38 males. Children completed the ADOS-Module 1, 2 or 3 and their parent completed the ADI-R with trained examiners. The ADOS assesses current behaviors while the ADI-R reviews developmental history by parental report and its algorithm emphasizes the age 4 to 5 years old. Of the 40 children, 35% made criteria for Autistic Spectrum Disorder on the ADOS algorithm and were also judged to be autistic clinically. Interestingly, 42% of the 40 children did make criteria on the ADI-R for autism. This percentage is higher than the 35% because the criteria for autism is related to behavior at age 4 to 5 years and several of the children with FXS improved by middle childhood and did not meet criteria when they were evaluated in the clinic. Those cases that appeared to improve dramatically from an earlier age will be described in terms of cognitive, language abilities, interventions received and molecular variables.

Phenotypic consequences in a child with a de novo X;1 translocation. *S. Yatsenko, T. Sahoo, M. Santini, J. Wu, F. Scaglia, R. Naeem.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

X chromosome inactivation is a process occurring in somatic cells of mammalian females that leads to the silencing of the majority of genes on one X chromosome and serves as a mechanism of X-linked gene dosage compensation between the sexes. It is a random event occurring early in embryogenesis and the molecular mechanism remains unclear. Individuals harboring structural abnormalities of the X chromosome, including X;autosome translocations, provide a valuable resource to characterize genotype-phenotype correlates in the context of X chromosome gene content and inactivation. In cases of unbalanced X;autosome translocation the derivative X is usually inactivated and the X inactivation spreads variably into an attached autosomal segment. Replication behavior of translocated autosomal segments indicates a delay in replication that may also be discontinuous, and is probably the reason for an attenuated phenotype in many cases. We report a case of X;1 translocation in a 9-month-old girl with mild dysmorphic features and developmental delay. High-resolution chromosome analysis showed a de novo unbalanced translocation between the chromosomes X and 1 [46,X,der(X),t(X;1)(q27;q31.2)]. Fluorescence in situ hybridization (FISH) using chromosomes X and 1 telomere-specific probes confirmed the finding. The genotype in the patient results in monosomy for Xq27-qter and trisomy for 1q31-qter. Patients with trisomy for the distal segment of 1q present a more severe phenotype compared to that seen in our patient and include facial dysmorphisms, urogenital and cardiac anomalies. The absence of manifestations typical of trisomy 1q suggests that the inactivation of the derivative X chromosome significantly attenuates the phenotype in our patient, which can be explained by the possible lack of inactivation of some segments of chromosome 1 on the derivative X. Interestingly, the deletion breakpoint on the X chromosome occurs within the fragile site at Xq27. Further studies to determine the role of X inactivation on the resulting phenotype, as well as association of X chromosome fragile site in X;1 translocation, will be important to characterize genotype-phenotype correlation.

Distal 10q deletion in a boy with developmental delay and heart defect using subtelomeric FISH probe. A.
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New cytogenetic telomeric probe sets have identified small chromosomal rearrangements involving the terminal bands (subtelomeric sequences) in approximately 4-7% of cases of previously unexplained mental retardation. We report a 16-month old boy with 10q26.3 deletion that was found using a subtelomeric probe kit (Vysis, Inc). His mother's karyotype was normal. Initial karyotype and FISH for 22q deletion at 6 months of age were normal (46,XY). The pregnancy history was significant for maternal ingestion of 2 cups of vodka per week prior to learning she was pregnant, and marijuana use. These were discontinued after her first missed menstruation. The parents are not consanguineous. He had a maternal half-sibling and three paternal half-siblings who were reported to be healthy. He was born at term by spontaneous vaginal delivery to a 21-year old mother and a 22-year old father. Birth weight was 2438 grams, birth length was 46 cm and head circumference was 32.5 cm. He had hypotonia, global developmental delay, poor growth, microcephaly, hypertelorism, long and flat philtrum, thin upper lip, small chin, low-set ears, left torticollis, left plagiocephaly, and fisted hands. He was s/p repair of total anomalous pulmonary venous return and atrial septal defect, grade 5 vesico-ureteral reflux with hydronephrosis, reactive airway disease, left esotropia, myopia, and mild gastro-esophageal reflux. Metabolic work-up was unremarkable. At 11 months, his weight, length and head circumference were below the third percentile. He was not sitting, he was not reaching purposefully for objects, and he did not have any speech. Telomeric probe set use will reveal new cytogenetic syndromes such as the present case.

Cytogenetic Approaches to Finding Auditory Genes. *R.E. Williamson¹, C.C. Morton^{1,2}*. 1) Harvard Medical School, Boston, MA; 2) Brigham and Women's Hospital, Boston, MA.

Hearing loss is a common sensory disorder with an estimated incidence of 1 in 1000 human births. Approximately half of cases are attributed to environmental factors, while at least 50% are due to genetic causes. Genes with a role in the auditory system have been identified through both genetic linkage studies of families with heritable deafness and positional candidate gene approaches. Another method for gene discovery is to ascertain deaf individuals that carry balanced translocations and identify disrupted or dysregulated genes at the site of chromosomal rearrangement. Here, FISH experiments were done to map the breakpoint regions on each of four derivative chromosomes in two deaf individuals. One case, designated DGAP090, has a translocation between chromosomes 8 and 9 [t(8;9)(q13;p22)]. These breakpoints were determined by karyotype and probes were chosen based on proximity to the affected region. Successive FISH experiments were then performed to identify a split BAC that hybridized to the normal chromosome as well as to both of the derivative chromosomes. Further refinement of the breakpoint was done using PCR products. The chromosome 8 breakpoint disrupts a hypothetical gene and, on chromosome 9, a known gene is located in the breakpoint region. The other case, DGAP056, carries a translocation between chromosome 2 and chromosome 13 [t(2;13)(p24;q21)]. Hypothetical genes appear to be disrupted by the breaks on both chromosomes 2 and 13. Confirmation of the disruption of these sequences at the breakpoints is currently underway using a variety of approaches including FISH with cDNAs where available and Southern blot analysis.

Mendelian cytogenetics: Fine physical mapping of a gene for autosomal recessive syndromic neuronal migration defect using homozygosity for a balanced reciprocal translocation. *L. Baala^{1, 2}, S. Briault³, F. Laumonnier³, J. Amiel¹, H. Natiq², A. Assermouh⁴, M. Vekemans¹, A. Munnich¹, A. Sefiani², C. Moraine³, S. Lyonnet¹.* 1) Genetique INSERM U393, Hopital Necker, Paris, France; 2) Institut National d Hygiene, Rabat, Maroc; 3) Service de genetique, INSERM U316, CHRU Bretonneau, Tours, France; 4) Hopital Enfants Avicenne, Rabat, Maroc.

We report on syndromic neuronal migration defect (NMD) in four patients belonging to a large inbred Moroccan family. Associated features included craniosynostosis, adducted thumbs, and severe psychomotor retardation. Three children died at 15, 18 and 36 months respectively. To our knowledge, this combination of features has not been reported before. Interestingly, this family segregates a balanced reciprocal translocation $t(3;10)(p24-q23)$, and the three affected family members originating from three family branches were found homozygous for the translocation while their parents were heterozygous. Inbreeding and absence of symptoms in the parents suggested an autosomal recessive mode of inheritance. When assuming a fully penetrant autosomal recessive disorder, linkage analysis between the disease phenotype and translocation breakpoints used as polymorphic markers yielded a maximum lodscore of $Z = 3.45$ at 3p24 or 10q23, when including three affected individuals only. Indeed, using homozygosity mapping, we identified a region homozygous by descent for each of the two chromosomes. These data prompted us to hypothesize that: i) the mutated locus for this novel syndromic NMD maps to either chromosome 3p or 10q, and ii) the translocation breakpoint disrupts a recessive disease-causing gene. Bacterial artificial chromosome (BAC) contigs encompassing the 3p and 10q breakpoints were constructed, and BACs crossing the breakpoints were identified for both chromosomes, defining minimal candidate regions of 200 kb and 52 kb in length respectively. Characterization of full-length cDNAs and ESTs in BACs clones overlapping on chromosomes 3p and 10q is underway, as well as a long-range PCR approach in order to determine whether a gene(s) sequence(s) is (are) altered by this familial reciprocal translocation.

Severe brain malformation associated to ring chromosome 22. *R. Linares¹, M. Orera², M.A. Palomar¹, C. Alonso³, J. Delcan¹, C. Lostau², E. Garcia Poblete¹, M.D. Lillo², S. G.Gomez de las Heras¹.* 1) Embriology, U. Rey Juan Carlos, Alcorcon, Madrid, Spain; 2) Genetics, H. Gregorio Maranon; 3) Clinical Analysis. H. de la Princesa.

INTRODUCTION: Ring chromosome 22 is a fairly uncommon cytogenetic abnormality, with some 50 cases published so far. The clinical features more frequently described include craneofacial anomalies, global developmental delay, hypotonia, unsteady gait and severe speech retardation, suggesting an alteration in neurological development. **MATERIAL AND METHODS:** We performed an amniocentesis in a 34 years old patient on her 16th gestational week because of maternal anxiety. The fetal karyotype was 46 XX, r22 in a non mosaic form. We also performed FISH analysis with WCP 22 (VYSIS) and a mix of probes for bands 22q11.2 and 22q13.3 . The results confirmed the presence of a ring chromosome 22 with no deletions of the above mentioned bands. The parental karyotypes were normal. The macroscopic external appearance of the fetus was normal. The thoracic and abdominal organs did not showed any apparent anomaly. The morphologic study of the encephalon showed total agenesis of the corpus callosum, septum pellucidum and fornix. The longitudinal cerebral fissure was covered in all its extension by glial epithelial cells. This cells have a ciliated border and therefore are identified as ependymal cells, of the type that usually cover the ventricular cavities and the ependymal conduct. We explain the presence of these cells covering the entire longitudinal cerebral fissure, because of the total absence of telencephalic comissures. **DISCUSSION:** We describe severe SNC malformations associated with non mosaic ring 22 chromosome complement. The total absence of telencephalic comissures hinders communication between both cerebral hemispheres. This circumstance could be interconnected with the etiopathogenic pathway of the neurological aspects of the syndrome. The development of telencephalic comissures is been connected with FGF8, EMX1 and GLI3. None of these genes is located in 22q13.3-ter, therefore we hypothesize the presence of a novel gene in this area involved in development of the telencephalon.

Deletion of the CNS Calcium binding protein CALN1 in WMS with high function. *G. Mohapatra¹, X.N. Chen¹, H. Wijesuriya¹, U. Bellugi², J.R. Korenberg¹.* 1) Med Genet, Cedars-Sinai Medical Center, UCLA, Los Angeles, CA; 2) The Salk Institute, San Diego, CA.

Williams syndrome (WMS) is a complex neurodevelopmental disorder due to the deletion of at least 17 genes on 7q11.23. Majority of the WMS patients have a similar de novo deletion suggesting that the deletion breakpoints fall within a defined physical region. Therefore, WMS subjects with partial deletions are now providing clues to explore the genes responsible for subsets of the neurocognitive phenotype. The Calneuron 1 (CALN1) gene maps to 7q11, about 1Mb upstream of the common WS deletion breakpoint and encodes a calcium-binding protein of the calmodulin superfamily, specific to the adult brain. Here, we report the deletion of the CALN1 gene in an atypical WMS subject, 5623, with high cognitive function. This was determined by using FISH, PCR of hybrid cell lines, Southern blot and familial genotyping. To determine the possible contribution of CALN1 deletion to typical WMS, we examined the deletion structure of a cohort of 41 WMS subjects, using a panel of BACs (815K3 CALN1, 1008H17 FZD9, 592D8 ELN and 1184P14 GTF2I), hybridized in groups of 3 to evaluate order and spacing. Of the 41 cases, only subject 5623 showed a deletion of BAC815K3 which contains the 5'UTR and 1st exon of CALN1 gene. We subsequently confirmed the CALN1 deletion by using PCR of DNA markers from the clones spanning the CALN1 gene in 5623 hybrid cell lines and localized the breakpoint between markers WI-21143 and D7S1776 located between exon 3 and exon 4 of the CALN1 gene. Genotyping of the 5623 family using 11 polymorphic markers from the typical WMS deletion and flanking regions revealed the deletion to be paternal in origin and confirmed the loss of D7S653 in 5623. Further analysis of the father's chromosome using BACs 815K3, 208H19, 1184P14 and 1129E22 by 4-color FISH, showed the father to be a carrier of an inversion polymorphism. The lack of CALN1 deletion in our WMS cohort suggests that the CALN1 deletion is not a major contributor to the WMS phenotype. However, the deletion of CALN1 in 5623 provides an unexpected opportunity to define the role of brain specific Ca²⁺ signaling in human cognition.

A locus for brain development maps to chromosome 6q26-qter. *N. Qin¹, B. White¹, E. Woolley², L. Duckworth¹, A. Anguiano¹, R.E. Schnur².* 1) Quest Diagnostics Nichols Institute, San Juan Capistrano, CA; 2) Childrens Regional Hospital at Cooper Hospital/Robert Wood Johnson Medical School, Camden, New Jersey.

A male infant was born with massive hydrocephalus and macrocephaly. Other growth parameters were normal. He had relatively minor dysmorphic features. These included small palpebral fissures, low set but normally formed ears, wide spaced nipples, sacral anomaly, and adducted thumbs. He had generalized hypotonia, a weak cry, and abnormal EEG. He also had optic atrophy. MRI showed massive hydrocephalus with a normal 4th ventricle, consistent with aqueductal stenosis. The cortical rim was very thin in the occipital, parietal and posterior frontal lobes. There was evidence of semilobar holoprosencephaly, with a monoventricle, fused thalami and absent septum pellucidum. Partial agenesis of the corpus callosum was also noted. Cytogenetic analysis revealed a subtle terminal deletion of the long arm of one homologue of chromosome 6 [karyotype: 46,XY,del(6)(q26)]. FISH using a 6q subtelomeric probe confirmed the suspected deletion. Parental chromosomes and FISH studies were normal. Therefore, the proband's deletion is apparently de novo, barring parental mosaicism. Occult trisomy for another chromosomal segment has not yet been fully excluded. Deletions of chromosome 6q with breakpoints distal to 6q25 are distinctly uncommon. Non-specific brain malformations and hydrocephalus, sometimes with aqueductal stenosis are associated with these deletions, as they are with ring (6) chromosomes. Our case with a variant of holoprosencephaly and aqueductal stenosis lends additional support to the localization of a gene critical to brain development that lies within the terminal 6q region.

Familial premature centromere division report: cell cycle studies and review of the literature. A. Corona-Rivera^{1,3}, T.A. Garca-Cobian¹, F. Salamanca-Gomez⁴, C. Palomino-Cueva¹, L. Bobadilla-Morales¹, J.R. Corona-Rivera^{1,2}, E. Corona-Rivera¹. 1) Laboratorio de Genetica Humana, Univ. de Guadalajara, CUCS, Guadalajara, Jalisco, Mexico; 2) OPD, Hospital Civil Juan I Menchaca, Guadalajara, Jal., Mexico; 3) OPD, Hospital Civil de Belen, Guadalajara, Jal., Mexico; 4) Unit of Investigation in Human Genetics, National Medical Center, IMMS, DF, Mexico.

Anaphasic figures, with all or almost all chromosomes with separated primary constrictions and splayed chromatids are rarely observed in lymphocyte cultures. High rates of anaphases (more than 5%), have been observed in at least ten previous family reports, referred as premature centromere division (PCD). We report a family in which four members of the paternal lineage, including the propositus with a MCA/MR syndrome, showed increased anaphases (8-36%) with no aneuploidies. Active centromeres were detected in the propositus and his parents by NORs stain. Statistical cell cycle time reduction (21.8h;SD0.4 in PCD individuals vs. 31.8h;SD3.9 in controls), and increased cell proliferation kinetic were observed in anaphasic cells of PCD individuals by differential sister chromatid analysis. FISH studies revealed the presence of alpha satellite DNA and CENP-B box sequences. Such findings emerges as distinctive features supporting cell cycle involvement in PCD trait. Autosomal dominant inheritance of PCD is confirmed. 38 out 47 PCD individuals previously reported have been phenotypically normal or with coincidental findings, however, abortion or infertility has been observed in 6 out 10 families. Two paternal aunts showed infertility in this family. We propose that increased spontaneous abortion and infertility is involved more than could be supposed when PCD is present.

Del 5p15.3 in a Family with Cat-like Cry and Developmental Delay. *D. LaGrave*¹, *P.L. Bader*², *J.K. Bader*², *X. Yang*¹, *B. White*¹. 1) Quest Diagnostics' Nichols Institute, San Juan Capistrano, CA; 2) Northeast Indiana Genetic Counselling Center, Ft. Wayne, In.

We describe a family consisting of a mother and her two children (two year-old female and newborn male), who all three share a terminal deletion at 5p15.3. They share features such as a cat-like cry in infancy, learning disabilities, hypotonia, small size at birth, and failure to thrive. They share mildly dysmorphic features as well, including a triangular face, malar hypoplasia, micrognathia, partial 2-3 syndactyly and curvature of the 5th finger and the 4th and 5th toes. Cytogenetic analysis on the mothers peripheral blood lymphocytes revealed a terminal deletion of the short arm of one chromosome 5, consistent with cri-du-chat syndrome. However, FISH analysis using a probe specific for the cri-du-chat critical region demonstrated a normal hybridization pattern, indicating the chromosome breakpoint was distal to the usual segment deleted in persons with cri-du-chat syndrome. Due to developmental delay in the proband's daughter and poor feeding in her son, cytogenetic testing (karyotype and FISH for cri-du-chat) was performed and revealed that both children had inherited the deletion from their mother. FISH for all subtelomeres was performed (probe grid, Cytocell, Ltd.) and confirmed a terminal deletion of 5p in both children. The signal for the SubTel 5p probe was not observed elsewhere in the children's karyotypes, ruling out the presence of a subtle rearrangement. The mother's SubTel 5p study confirmed that she also carried a true deletion. Karyotypes of peripheral blood lymphocytes done on the proband's parents were normal, indicating the proband's deletion of 5p was *de novo*. In summary, we describe a family with an unusual terminal deletion of 5p in that they demonstrate many of the symptoms of cri-du-chat syndrome, including the characteristic cat-like cry, but are not missing the critical region for cri-du-chat. Mental outcome appears to be much better than in patients with the typical cri-du-chat deletion with only mild to moderate developmental delay and eventual learning disabilities.

Partial deletion of the common 1,5 Mb critical region in an infant with classical Williams-Beuren syndrome. A. Winterpacht¹, A. Rauch¹, S. Endele¹, B. Schroeder², C. Steglich², S. Luetzgen², R. Heller². 1) Institute of Human Genetics, University of Erlangen-Nuremberg, Erlangen, Germany; 2) Institute of Human Genetics, University of Hamburg, Hamburg, Germany.

Williams-Beuren syndrome (WBS; OMIM 194050) is a contiguous gene deletion disorder with a variable clinical phenotype that is caused in most cases by a heterozygous microdeletion in 7q11.23. Due to two highly homologous flanking ~300 kb duplicons, the microdeletion is usually of similar size in almost all cases and encompasses a common ~1,5 Mb interval that contains at least 17 genes mostly of uncertain pathogenetic relevance. Phenotype-genotype correlation studies for WBS are hampered by the uniform size of the microdeletion. Here we report the case of a 1-year-old boy with a full WBS-phenotype that is caused by a partial deletion of the common ~1.5 Mb interval. Initial analysis with two sets of commercially available FISH-probes (Appligene/Oncor and Vysis) yielded conflicting results. We therefore carried out deletion mapping with microsatellite markers and an array of targeted FISH probes. We mapped the proximal and distal breakpoint of the deletion on two BAC clones. The deletion spans a region between the elastin gene (*ELN*) and the distal duplicon and therefore excludes *STX1A* and *FZD9* from the WBS minimal critical region of deletion. Fine mapping and sequencing of the breakpoints is in progress. Evidence from two similar cases in the literature also suggests that the centromeric portion of the 1,5 Mb interval is not always deleted in patients with full WBS-phenotype, defining a WBS critical region estimated to be less than 1 Mb. The conclusions from our work concern diagnosis and molecular etiology of WBS: 1. Quantitative differences in signal intensity after FISH analysis with commercial probes for a WBS microdeletion should be followed up carefully if partial deletions are not to be missed. 2. Even in patients with full WBS-phenotype the underlying molecular defect may be more variable than previously thought.

Chromosome instability induced in vitro with mitomycin C in five Seckel syndrome patients. *L. Bobadilla-Morales¹, A. Corona-Rivera^{1,2,3}, J.R. Corona-Rivera^{1,2,3}, T.A. Garcia-Cobian¹, D. Garcia-Cruz^{2,4}, E. Corona-Rivera¹.*
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The Seckel syndrome (SS) is an autosomal recessive entity with short stature and prenatal onset microcephaly, bird-headed profile, and severe mental delay. Hematological anomalies have been observed in around 25 % of the patients. Chromosome instability induced by mitomycin C (MMC), has been observed in previous reports. The purpose of this work is to report cytogenetic features in five patients with SS whose lymphocyte cultures were induced with MMC. The patients had low birth weight (mean 1870g), mental retardation, dwarfism (DS,-7.9), short stature (DS,-6.36), microcephaly (OFC;DS,-8.1), bird-like face and multiple articular dislocations. None of them had anemia at the time of examination. In all cases their parents were healthy and non-consanguineous. Lymphocytes of SS patients and a control group (n=9) matched by age and sex, were cultured with and without MMC, added 24 hs after the setup and harvested at 72 and 96 hs. Chromosome aberrations (chromatid and chromosomal gaps and breaks, deletions, fragments, and exchanges), were scored in 100 metaphases per culture. Sister chromatid exchanges method was also performed with no differences between both groups. Statistical increase of chromosome aberrations was observed in 96 hs MMC cultures in the five patients (40.2 vs 2.8 %), meanwhile in 72hs MMC cultures, such difference was observed in one patient (24 vs 4 %). The fact that patients with SS present chromosome instability induced with MMC, strongly support the existence of subgroups with pancytopenia as previously proposed and those without hematological disorders as our cases, since genetic heterogeneity has been suggested. The presence of triradial and tetradials in two patients suggest SS and Fanconi pancytopenia relation. Induced MMC chromosome instability in SS patients is a feature that must be ascertained in future studies.

Twin brothers with MIDAS syndrome and XX-male karyotype. *A. Anguiano¹, X. Yang¹, J.K. Felix², J.J. Hoo³.* 1) Cytogenetics Dept, Quest Diag, Nichols Inst, San Juan Capist, CA; 2) Pediatrix Medical Group, Inc., Arnot Ogden Medical Center, Elmira, NY; 3) Department of Pediatrics, SUNY Upstate Medical University, Syracuse, NY.

Twin brothers with microphthalmia, facial dermal hypoplasia, sclerocornea, and supraventricular tachycardia, are reported. Their clinical features are compatible with MIDAS syndrome, a known X-linked and hemizygous male lethal condition. Their karyotypes showed an XX sex chromosome modality with a subtle Xp/Yp translocation proven by the presence of SRY gene. The pregnancy was complicated with fetal supraventricular tachycardia, which was treated with digoxin prenatally. Postnatally, both twins required treatment with adenosine, digoxin and propranolol to remain in normal sinus rhythm. The possible involvement of the heart, only in the form of cardiomyopathy with arrhythmia is emphasized. Peripheral blood samples from each twin were submitted for a conventional GTG cytogenetic study. Both revealed an apparently normal 46,XX karyotype. Since these twins have a male phenotype with the suspected MIDAS condition, subsequent FISH assays revealed a positive signal for the SRY probe, and lack of hybridization in one X chromosome of the probes in the Kallmann and in the Steroid Sulfatase Probe (STS) loci at the terminal region of Xp. Replication studies with R banding, imaging storage and subsequent FISH assays in which SRY and STS probes were used on the same slides, demonstrated that the X chromosome bearing the SRY region also had a deletion of the STS probe. The chromosome X with the rearrangement at Xp22.3 was documented as invariably late replicating (X inactivated) in all 20 metaphases that were examined for each patient. In this rearranged X chromosome, the Xp terminal region consistently showed a dark contrast in the R banding study, indicating that this region escaped X inactivation, as normally happens in the pseudoautosomal region of the X chromosome. To our knowledge, this is the first report of twin brothers having MIDAS syndrome and XX-male karyotype.

Molecular cytogenetic characterization of mosaic and partial microduplication 21qter associated with non-specific mental retardation. *J.J. Lespinasse¹, C. Ucla², T. Billette de Villemeur³, M.J. Brunel¹, S. Fert-Ferrer¹, C. Paravy¹, M.O. Rethoré⁴, S. Deutsch², S. Dahoun², A. Reymond¹.* 1) Lab Cytogenetics, General Hospital, Chambéry Cedex, France; 2) Division of Medical Genetics, University of Geneva Medical School, Geneva, Switzerland; 3) Service de Neuropédiatrie, Hôpital Armand Trousseau, 75571 Paris, France; 4) Centre Médical Jérôme Lejeune, 75116 Paris, France.

We report a patient with autistic disorder and partial microduplication of chromosome 21qter without features of Down Syndrome. The patient, a 20-year-old female, was reported to have severe mental retardation and discrete clinical features including blue sclerae, Brushfield spots, thin upper lip, webbed neck. High-resolution chromosome analysis (GBTG, RTBG) from peripheral blood lymphocytes revealed a 46,XX constitution. Fluorescence in situ hybridization (FISH) with subtelomeric commercially available probes (Vysis and Oncor) suggested a mosaic with a distal 21q22.3qter duplication. Her karyotype was 46,XX,ish dup(21)(q22.3qter)(Tel21qx2, D21S1219x2, D21S1220x2)[50]/21(q22.3qter)(Tel21qx2, D21S1219x2, D21S1220x2)[50], while her parents were karyotypically normal. The patient karyotype was confirmed by quantitative SNPs analysis using the Pyrosequencing technology (Pyrosequencing). This type of chromosome anomaly is never reported in the literature to our knowledge. Further studies are required to confirm a causal relationship of 21qter microduplication and psychotic disorder.

Familial Partial Duplication (1)(p33p22.3). *R.L. Young¹, T.F. Thurmon¹, H. Chen¹, M. Nordberg², J.A. Bocchini, Jr¹.*
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At birth, the proband's head circumference was 35 cm (58th percentile), length 51 cm (63rd percentile), and weight 3562 gm (58th percentile). He had redundant neck skin, low-set ears with simple architecture, close-set eyes, broad nose with upturned tip, retrognathia, midline posterior cleft palate, and small hands and feet. A bridging crease joined his two palmar creases. Echocardiography showed a small muscular ventricular septal defect, patent foramen ovale, small ductus arteriosus, and mild insufficiency of the mitral and tricuspid valves. Spine x-ray showed poorly developed cervical vertebrae. Renal ultrasound showed crossed fused ectopia of the kidneys (horseshoe kidney). Postnatal swallowing dysfunction required a feeding gastrostomy. BMI has remained over 50th percentile, but growth and development parameters were below 3rd percentile at 1 year of age. Chromosome analysis with GTG banding at 500 band level from a commercial laboratory was interpreted as 46,XY,duplication(10)(q11.21q22.1). We noticed that the mother's 42-year-old half-sister was visibly similar, had poor mental function and was small: Head circumference 50 cm (less than 1st percentile), height 153 cm (2nd percentile), and weight 47.27 kg (3rd percentile). Our GTG banded karyotype showed a chromosome 10 that appeared identical to the proband's, but chromosome-specific FISH indicated that the duplication was from another chromosome. Karyotypes of the mother, maternal uncle and maternal grandmother showed a balanced rearrangement in which bands p33p22.3 of chromosome 1p had been inserted into chromosome 10q. The proband and his maternal aunt had only the 1p insertion into 10p. In the duplicated segment, McKusick's morbid anatomy of the genome lists 26 disease-related genes that might be expected to show dosage effects. To date, neither of the affected patients has displayed evidence of any of them. Two other reports provided data to suggest familial transmission of a balanced insertion of part of 1p but our case is the first clearly documented one. In those reports, the segment was inserted into different chromosomes, possibly reflecting areas of duplication of 1p.

Mapping the Wolf-Hirschhorn syndrome phenotype outside the currently defined Wolf-Hirschhorn syndrome

critical region. *M. ZOLLINO¹, R. Lecce¹, R. Fischetto², A. Selicorni³, F. Faravelli⁷, M. Murdolo¹, C. Butt⁴, G. Capovilla⁶, L. Memo⁵, G. Neri¹.* 1) Institute of Medical Genetics, Universit Catt. Sacro Cuore, Rome, Rome, Italy; 2) Divisione di Malattie Metaboliche e Genetiche. A.O. di Venere-Giovanni XXIII, Bari; 3) Clinica Pediatrica Universit di Milano; 4) IRCCS E. Medea Associazione La Nostra Famiglia, Bosisio Parini, Lecco; 5) U.O. Patologia Neonatale Ospedale Ca' Foncello, Treviso; 6) Neuropsichiatria Infantile A.O. Carlo Poma, Mantova; 7) Ospedali Galliera, Genova, Italy.

Wolf-Hirschhorn syndrome (WHS) is a contiguous gene syndrome resulting from partial 4p deletion. The currently considered WHS critical region (WHSCR) is restricted to the 165 kb interval in 4p16.3 defined by the loci D4S166 and D4S3327. Three WHS candidate genes have been independently described: WHSC1 and WHSC2 fall within the WHSCR, LETM1 flanks, being outside, the WHSCR. We analyzed a total of 9 patients carrying a terminal 4p16.3 microdeletion. Phenotypically, all of them presented with typical facial appearance, mild mental and growth retardation and seizures. Congenital malformations were absent, head circumference was normal in two occasions. Although genotype-phenotype correlations in WHS mostly depend on the deletion size, we found that the shared WHS phenotype is defined by the association of all the following signs: typical facial appearance, mental retardation, growth retardation and seizures. Genetically, the deletion varied in size from 1.3 to 2.4 Mb. The currently defined WHSCR was fully preserved in the patient carrying the 1.3 Mb deletion, that included LETM1. The unique reported patient with an interstitial deletion restricted to the WHSCR presented with a quite atypical WHS phenotype, lacking growth retardation and, more importantly, with no seizures. The few reported interstitial deletions, preserving LETM1, also resulted in an atypical WHS phenotype, lacking seizures. On our observations, the distinctive WHS phenotype maps distally, and it is completely outside the currently defined WHSCR. LETM1 turns to be the strongest candidate gene for seizures in this condition.

Cytogenetic Expression of Fragile Site Belong X Chromosome (FRAXA) in Patients with Speech Delay and Normal or Borderline IQ. *M. Diaz-Garcia, G. Garcia Sanchez.* Laboratorio de Genetica, InCH/CNR.

Fragile X syndrome is the most common inherited form of mental retardation. The syndrome is characterised by moderate to severe mental retardation, macroorchidism, large ears, prominent jaw and speech delay. In the Instituto de Comunicacion Humana of Mexico, we decided to search for the presence of FRAXA in a population of 433 individuals with normal intelligence (IQ) or borderline (70-80) and speech delay. The patients were selected through consultation of genetics to them realised their clinical history and diagnosis of psychological, speech, audiology etc. We performed chromosome analysis through three induction methods in order to identify FRAXA, were analysed per patients 300 metaphases. All the patients were diagnosed with speech delay: preservation, echolalia, jargon, stutter and articulation and omission errors. The most patients presented characteristics of common behaviour: emotional lability, short lapses of attention and low tolerance to the frustration. The majority of patients had been present alterations in one or both ears and others alterations phenotypic common at the syndrome. All the patients had antecedent hereditary of speech delay. In the chromosome analysis 422 were negative FRAXA and 7 positive FRAXA with different percentage in the expression. However with the application of the cytogenetic techniques we can't identify the carrier FRAXA and may be in some patients the alterations it is masked, whereby it requires the use of methodology applied in Molecular Biology to detect permutation or full mutation of trinucleotide repeat expansion of the FMR1 gene.

Subtelomeric FISH detects cryptic anomalies in a family with a boy with chimerism and a large balanced t(4;5).

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Testing for cryptic abnormalities by the use of subtelomeric FISH probes has become an important diagnostic tool in the work up of patients with mental retardation, dysmorphic features and congenital malformations. In such cases about 3 % of subtelomeric abnormalities appear to be inherited from one of the parents carrying a balanced chromosomal rearrangement. We report a 3-month-old boy who was diagnosed with ambiguous genitalia, dysmorphic features and developmental delay. Standard chromosome analysis on blood revealed a chimeric karyotype of 46,XY,t(4;5)(q31.1;q14)[46]/46,XX[4]. The boy had intra-abdominal gonads that were testicular by biopsy. He presented with multiple dysmorphic features, marked hypotonia, developmental delay, poor growth and relative macrocephaly. Physical exam of his 2.5-year-old sister also indicated hypotonia, significant developmental delay, relative macrocephaly and the same dysmorphic stigmata. In addition, she had several congenital malformations, including pulmonary valvular stenosis and pineal cyst. Her karyotype was found to be 46,XX. Due to the similar phenotype of the siblings, subtelomeric FISH studies were performed. In addition to the known karyotypic abnormalities, the boy was found to have a derivative chromosome 5 with 5pter deletion and a 17pter duplication, which was also detected in his sister. FISH probes for the critical cri du chat region on 5p15.2 were present in both siblings. Whereas the mother's karyotype was normal, chromosome analysis of the father revealed a subtle balanced t(5;17)(p15.31;p13.1) confirmed by subtelomeric FISH. Analysis of this interesting family demonstrates the utility of subtelomeric FISH in the diagnosis of cryptic chromosome anomalies in patients for whom previously identified chromosomal findings appear insufficient to explain the phenotypic anomalies observed.

Cytogenetic Evaluation of a Hormonal Drug Mestranol in Human Lymphocytes In vitro. *M.E. Ahmad¹, G.G.H.A. Shadab², M.A. Azfer²*. 1) Anatomy, AIIMS, New Delhi, India; 2) Zoology, AMU, Aligarh, UP, India.

Cytogenetic evaluation of a commonly used synthetic estrogen, Mestranol, was carried out in human peripheral blood lymphocytes in vitro. Chromosomal aberrations (CA) and sister chromatid exchanges (SCE) were used as genetic end points. Final concentrations of the drug were determined after observing its effects on Mitotic Index. The study was carried out both in the presence as well as in the absence of rat liver metabolic activation system (S9 mix). The lymphocytes were exposed to three different concentrations of the drug i.e. 10, 25 and 50mg/ml for three different durations i.e. 24, 48 and 72 hours. The drug was found to affect the genetic material both in the presence and absence of S9 mix. It induced chromosomal aberration and increased the SCE frequency at significant level in human lymphocyte chromosomes. In the absence of metabolic activation, maximum value for CA (27.00%) was observed at 25mg/ml concentration for 72h exposure duration. Further with an increase in the concentration this value decreased to an insignificant level in comparison to the normal control value (3.00%) particularly at higher durations, probably due to the death of cells. Again in the presence of S9 mix, maximum value for CA (29.50%) was observed at 25mg/ml concentration which was highly significant statistically in comparison to the normal control value. The drug Mestranol increased the SCE frequency in the presence as well as in the absence of S9 mix at 25mg/ml to a significant level (5.38 ± 0.180 and 5.04 ± 0.224 respectively) and the frequency decreased with an increase in the concentration. Thus it was concluded that the drug Mestranol and possibly its metabolites are genotoxic beyond a particular concentration in human lymphocytes.

Prader-Willi syndrome resulting from an unbalanced translocation: characterization by array CGH. P.D.

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The etiology of Prader-Willi syndrome is variable. The majority of cases are the result of microdeletions in proximal chromosome 15. The remainder are the result of maternal uniparental disomy of chromosome 15, imprinting center defects, and balanced or unbalanced chromosome rearrangements involving chromosome 15. We report a patient who presented with multiple congenital anomalies including microcephaly and dysmorphic craniofacial features of a high, posteriorly slanted forehead, hypoplasia of the supraorbital ridges, epicanthal folds bilaterally and a short nose with a depressed nasal bridge and bulbous tip. The patients ears were mildly low-set with thickened, overturned superior helices and prominent antihelices. His mouth had down-turned corners, a thin tented upper lip and he was retrognathic. He had scrotal hypoplasia with bilateral cryptorchidism. Neurologically, he was severely hypotonic and had mild contractures at the knees, bilaterally. Cytogenetic analysis showed a *de novo* 45,XY,der(5)t(5;15)(p15.3;q13),-15 karyotype. In effect, the proband had monosomies of 5p15.3→pter and 15pter→15q13. Methylation PCR analysis of the promoter region of the *SNRPN* gene showed only the maternal allele, consistent with the PWS phenotype. Similar to other patients with PWS resulting from unbalanced translocations, the proband's phenotype is more severe, also reflecting the contributions of the associated monosomies. Array CGH confirmed deletions of both distal 5p and proximal 15q and provided more accurate information as to the size of the deletions. This case illustrates the utility of array CGH in characterizing structural chromosome abnormalities.

A dic(19) chromosome with an interstitial duplication of 19p13.1 in a patient with hypotonia and mild dysmorphic features. *D.B. Flannery, S.M Delaney, A.S Kulharya.* Dept Pediatrics, Sect Genetics, Medical Col Georgia, Augusta, GA.

Stable dicentric chromosomes are rarely seen as constitutional chromosome abnormalities. The majority of dicentric chromosomes in humans occur as marker chromosomes derived from chromosomes 15 or 22 or as Robertsonian translocations. For a dicentric chromosome to be mitotically stable through successive cell divisions, the centromeres have to be either very closely situated so as not to disrupt the spindle formation or one of the centromere becomes latent or inactive. We present a case with an unusual abnormality of chromosome 19. The patient is a two-year old male who was evaluated for hypotonia and dysmorphic features. Chromosome analysis demonstrated a male karyotype with additional material on the short arm of a chromosome 19. FISH with a panel of whole chromosome paint probes and subtelomeric probes showed that the additional material was derived from chromosome 19 itself. The presence of a very dark G-band similar in intensity to that of the centromere was not compatible with a direct or inverted duplication of 19p. C-banding demonstrated a C-positive band at 19p13.1 suggesting the presence of a second inactive centromere. The parental karyotypes were normal. This patient therefore has a small duplication of the p13.1 region resulting from a dicentric chromosome. Duplications and deletions of chromosome 19 are very rare for both arms of this chromosome. Two cases of partial trisomy of 19 are reported that resulted from abnormal segregation of a balanced translocation. Only one case of partial trisomy resulting from interstitial duplication of 19p has been reported. The interstitial duplication seen in our patient has not been reported previously. Several mechanisms may lead to the formation of a dicentric chromosome. These include meiotic recombination within a paracentric inversion loop, isochromatid break with U-shaped rejoining, and mitotic recombination. The small size of the second centromere implies that the stability of this abnormal chromosome in our patient may be due to the deletion of most of the active centromeric sequences in the second centromere.

Ring Y chromosome and several cell lines in a patient with growth delay and gonadal dysgenesis. M.

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Ring Y chromosomes are unstable during cell division. Therefore, most reported patients are mosaics, usually including a 45,X cell line. An uneven number of sister-chromatid exchanges in the monocentric ring chromosomes leads to dicentric ring chromosomes. Depending on the breakpoints (proximal or distal to SRY) and the level of mosaicism, the phenotype of the ring Y patients vary from normal males or females to intersex individuals and sex ambiguities. We present here the case of a 19 year-old male who was referred, at age 11, for growth delay. Other clinical features included hypospadias, cryptorchidism, and gonadal dysgenesis. Karyotypes on the left (96%) and right (62.5%) testicular tissue showed two cell lines: a predominant 45,X and 46,X,r(Y)(p11.3q12). The GTG-banded karyotypes on PHA-stimulated lymphocytes showed the same cell lines, the ring Y chromosome being seen in 76% of the metaphases analyzed. FISH done 8 years later with probes SRY (Sex Region of the Y chromosome), 91H4.5 (recognizing Yp11.2), DYZ3 (specific for the centromeric region of the Y chromosome), and DYZ1 (recognizing Y heterochromatin in Yq12) showed an increase in the 45,X cell line (60.6%) and a reduction in the monocentric 46,X,r(Y) cell line (35.5%). Of the 259 metaphases analyzed by FISH, three more cell lines were found: one with a double dicentric ring (8 cells), one with two double dicentric rings (1 cell), and another one with a quadruple tetracentric ring (1 cell). FISH with the all-telomeres probe was negative, thus the formation of the ring involved breakpoints at both ends of the Y chromosome. Since probes SRY and DYZ1 were positive, the breakpoints occurred in Yp11.3 (distal to SRY) and Yq12. Thus, very few euchromatic material has been lost. Therefore, we propose that the clinical manifestations of the patient is more likely to be the result of the rising 45,X cell line rather than to the deletion of the Y chromosome (This research was supported by grants from Reseau de Medecine Genetique Appliquee-FRSQ).

Craniosynostosis and MCA/MR with 7p microdeletion: evidence for a second locus in 7p15. *P. CALLIER¹, J. PUECHBERTY², P. BLANCHET², G. LEFORT¹, F. PELLESTOR³, P. SARDA².* 1) Cytogenetics Laboratory, CHU Arnaud de Villeneuve, Montpellier, France; 2) Genetics Unit, CHU Arnaud de Villeuve, Montpellier, France; 3) Institute of Human Genetics, CNRS UPR 1142, Montpellier, France.

Craniosynostosis as a part of Multiple Congenital Anomalies and Mental Retardation (MCA/MR) are often associated with chromosomal microdeletion in particular of the chromosome 7p. To date, only a few molecular studies have been performed in order to characterize the area of chromosome 7 involved in the non syndromic craniosynostosis. High-resolution analysis have suggested subbands 7p21-22 as the craniosynostosis locus region (CRS1) and a probable second locus (CRS2) located in the more proximal region. We report the study of two new cases of children affected by craniosynostosis associated with MCA/MR (short stature, dysmorphic facial traits, malformed small ears, cardiac defect, skeletal changes, mental retardation) and subtle deletion of the chromosome 7p. Molecular cytogenetic investigations, using YACs and BACs probes (locus D7S1895, D7S1936, D7S1438, D7S2369, D7S632), have been performed on cytogenetics preparations from these two children. In the first patient, a deletion has been evidenced in the region 7p15.2- 7p21.2. The gene "Twist" is localised in this domain. The second patient displayed a smaller deletion 7p15.1 - 7p15.3. This region could be candidate for a second craniosynostosis locus region CRS2 in 7p15 region.

Excess of the short arm of chromosome 22 resulting in a duplication 22q13.3 in a girl with severe growth retardation: a possible pitfall in cytogenetics. *D. Genevieve¹, P. Sachs³, C. Borie¹, O. Dupuy¹, M. Raoul², M.L. Jacquemont², Y. Sznajer², C. Pignal¹, A. Thaly¹, M. Vitu¹, C. Baumann², P. Evrard³, A. Verloes², P. Eydoux¹.* 1) Unite de cytogenetique; 2) Unite de genetique clinique; 3) Service de neurologie pediatrique, Hopital Robert Debre, Paris, France.

Duplication 22q13 resulting in segmental trisomy 22 has been rarely reported. The phenotype of the patients carrying such duplications depends on the chromosome bands involved. Patients with a proximal duplication q11-q12, or with a trisomy 22, have a phenotype resembling cat eye syndrome. Duplication q12-q13 results in a different phenotype. We report on a girl born at 36 WG to unrelated healthy Indian parents. Caesarean section was performed because of IUGR and abnormal fetal rhythm. Birth weight was 1960 g (-2 SD), birth length was 40 cm (- 3 SD) and OFC was 31 cm (- 2 SD). Severe feeding difficulties were noted in the neonatal period. At age 3, there was severe mental retardation, growth retardation, microcephaly, facial dysmorphism, small hands and feet and an ASD. Blood karyotyping showed an excess of the short arm of chromosome 22, identified as a duplication 22q13.3 with subtelomeric FISH analysis. Only 6 cases with pure trisomy 22q13.3 have been reported. Reviewing these reports and our case, we conclude that patients with duplication 22q13.3 may have a recognizable phenotype, namely severe mental retardation, pre and post natal growth retardation, microcephaly, facial dysmorphism and visceral malformations. A putative growth regulatory gene may account for intra-uterine and post-natal growth retardation in duplication 22q13.3 and overgrowth in the microdeletion 22q13.3 syndrome. Additional euchromatic material on the short arm of acrocentric chromosomes may be easily mistaken for a variant. A careful conventional and FISH analysis is warranted in case of such findings.

Nondisjunction studies in trisomy 13. *M. Bugge*^{1,2}, *M.B. Petersen*^{2,3}, *M. Bak*¹, *C.A. Brandt*³, *J.M. Hertz*³, *L. Tranebjaerg*³, *C.D. deLozier-Blanchet*³, *G.B. Petersen*³, *C. Lundsteen*³, *J. Lespinasse*³, *K. Rasmussen*³, *M. Mikkelsen*².
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The origin of nondisjunction in human trisomy 13 has previously been studied using DNA polymorphisms, but in a very limited number of cases and it has not been possible to determine the parental origin in all cases. We have investigated the parental origin of the additional chromosome in 57 cases of free trisomy 13 using 18 microsatellite DNA polymorphisms covering the length of chromosome 13q. The 57 cases were 29 prenatal, 26 postnatal, and 2 spontaneous abortions. The karyotypes were 23 of 47,XX,+13 and 34 of 47,XY,+13. In 50 cases the additional chromosome was of maternal origin, and in 7 cases of paternal origin. The addition of more DNA markers will enable the study of altered recombination associated with chromosomal nondisjunction, as previously described in other trisomies.

Functional disomy of distal Xq due to a der(Y)t(X;Y) in a male with severe congenital anomalies. *J.M. Gastier^{1,2}, I. Calloway¹, M.T. Millard¹, M. Ross¹, A. Thomas¹, P. Baker², J.D. Iams³, J. Atkin^{4,5}, G.D. Wenger^{1,2}.* 1) Dept of Laboratory Medicine, Columbus Children's Hosp, Columbus, OH; 2) Dept of Pathology, Ohio State Univ, Columbus, OH; 3) Division of Maternal Fetal Medicine, Ohio State Univ, Columbus, OH; 4) Division of Molecular and Human Genetics, Columbus Children's Hosp, Columbus, OH; 5) Dept of Pediatrics, Ohio State Univ, Columbus, OH.

Functional disomy of the X chromosome is known to be associated with severe phenotypic abnormalities, most commonly in association with a small ring X chromosome lacking XIST. These females present with additional features beyond a Turner syndrome phenotype. Rare cases of males with a duplicated X have been reported, frequently due to a recombinant pericentric inversion or inherited from the mother. We report an extremely rare case of nearly pure functional disomy of the distal long arm of the X chromosome due to a derivative Y chromosome arising from an X;Y translocation. Chromosome analysis was performed on amniotic fluid from a 20 week, 5 day fetus with ultrasound abnormalities including diaphragmatic hernia, abnormal spine, and thoracic mass. Maternal serum screening indicated a 1:14 risk for trisomy 18. Prenatal Aneuvysion FISH and testing for Smith-Lemli-Opitz Syndrome showed no abnormalities. G banding revealed pale material on the Y chromosome. FISH and molecular analysis confirmed the presence of the SRY locus on Yp and absence of the heterochromatin on distal Yq. FISH analysis with subtelomeric probes for Xp/Yp and Xq/Yq showed a normal hybridization pattern on the Y chromosome, suggesting an interstitial deletion of the Y chromosome or addition of distal Xq material. FISH using a whole chromosome paint confirmed that the material is derived from the X chromosome. The karyotype was described as 46,X,der(Y)(t(X;Y)(q27.3;q11.2)). The infant was delivered at 38 weeks and expired soon after birth. Clinical findings included hypoplastic heart, lungs, and ribcage. Radiological findings were similar to spondylothoracic dysplasia. To our knowledge, this is the first case of functional disomy for distal Xq due to a t(X;Y).

Association of a balanced autosomal translocation, t(1;12) (q34.1;q22) with mental retardation in a Han Chinese family from Taiwan. *C.H. Chen*^{1,2}, *C.C. Hung*², *K.F. Lee*², *J.S. Fang*². 1) Dept Psychiatry, Tzu-Chi General Hosp, Hualien City, Taiwan; 2) Institute of Human Genetics, Tzu-Chi University, Hualien City, Taiwan.

Mental retardation is characterized by the delayed and suboptimal development of general cognitive function, which is usually defined by the below-average of intelligence quotient. Mental retardation affects approximately 2-3% of general population, and its etiology is multifactorial. Genetics plays an important role in the etiology of mental retardation, and several genes involved in the syndromic and non-syndromic mental retardation have been identified. We identified a Han Chinese family from Taiwan with mental retardation through a referred child for evaluation of mental insufficiency. In this family, the mother is moderately mentally retarded, while the father is normal. They are not consanguineous. Two of their three boys were also found to have moderate mental retardation. No physical abnormalities were found in the mentally retarded individuals. Cytogenetic studies revealed that all three affected subjects have a balanced reciprocal autosomal translocation, t(1;12) (q34.1;q22). This translocation was found in the father and their normal child. The translocation was further confirmed by fluorescence in situ hybridization (FISH) using probes from chromosome 1 and 12. This chromosomal translocation associated with mental retardation in this family is a novel one, not reported in the literature. Further molecular studies of cloning the breakpoint sequences, and characterization of its effect should bring new insight into the mechanism of mental retardation in this family.

Phenotypic and Molecular Cytogenetic Characterization of a Girl with Mosaicism for Terminal Deletions of Chromosome 5p. *K.M. Allan¹, M. Perle², J.G. Pappas¹*. 1) Human Genetics Program, Department of Pediatrics, New York Univ Sch Medicine, New York, NY; 2) Cytogenetics laboratory, Department of Pathology, New York Univ Sch Medicine, New York, NY.

We describe an three-month old girl with a karyotype 46,XX,del(5)(p14)[14]/46,XX,del(5)(p15.1)[6] and Cri-du-Chat syndrome. She presented with weight, height and head circumference between the 3rd and 10th percentile for age. The clinical examination revealed downslanting palpebral fissures, broad nasal bridge, hypertelorism, epicanthal folds, micrognathia and cat like cry. The family history was not contributory. The peripheral blood karyotype detected mosaicism for two cell lines, each with a different deletion of part of the short (p) arm of chromosome 5; no normal cells were seen. Molecular cytogenetic (FISH) studies were also performed using in situ hybridization with fluorescent-labelled chromosome specific DNA probes for the short arm of chromosome 5 band p15.2 and the 5p telomere (Oncor, Inc D5S23 chromosome 5p15.2-specific probe and VYSIS, Inc TelVysion 5p telomere probe). Analysis of metaphase cells was consistent with the G-banding analysis and confirmed the loss of regions 5p15.2 and the telomere of chromosome 5p in each cell line. To our knowledge this is the second report of mosaicism for two cell lines each with del(5p) (P.C. Mainardi et al, J Med Genet 2001, 38: 151-158). The breakpoints in our case are different from the published case. Recent genotype-phenotype correlation studies have defined two deletion critical regions, one for the major clinical features of Cri-du-Chat syndrome in 5p15.2 and the other for the cat cry in 5p15.3. Both these regions are deleted in the two populations of cells identified in this patient; this is consistent with the clinical presentation.

Chromosomal damage frequency in hospital personnel exposed to low levels of ionizing radiation. *I. Ceja-Andrade*¹, *A. Corona-Rivera*^{1,2}, *T.A. Garcia-Cobian*¹, *L. Bobadilla-Morales*¹, *J.R. Corona-Rivera*^{1,4}, *A. Aceves-Escarcega*³, *E. Corona-Rivera*¹. 1) Laboratorio de Genetica Humana, Universidad de Guadalajara, CUCS, Guadalajara, Jalisco, Mexico; 2) OPD, Hospital Civil de Belen, Unidad de Citogenetica; 3) Instituto Jalisciense de Cancerologia (IJC), SSA; 4) OPD, Hospital Civil Juan I Menchaca, Div. de Pediatia.

Occupationally exposed personnel to ionizing radiation (OEP), may behave an increased potential risk of genomic damage. Spontaneous and induced chromosome aberration biomonitoring to evaluate low level of ionizing radiation effect is still open to discussion. The aim of this work is to evaluate chromosomal aberrations frequency in hospitalary OEP exposed to less than 30 mSv scored per year of ionizing radiation. Six POE working at radiotherapy or nuclear medicine departments for at least one year at Hospital Civil de Belen and IJC were included. Controls were matched by age and sex. Questionnaire and consent were fulfilled by them. Chromosomes were obtained by lymphocyte cultures from basal and 1.5 Gy radiated samples. At least 250 cells were scored per individual. Basal chromosome aberrations were increased in OEP (5.25%; 61/1161) vs. controls (1.07%; 19/1500. $P=0.04$ Wilcoxon test), mainly chromosome and chromatid breackages (31/1161) and tetradials (19/1161). Statistical increase was observed in basal versus radiated samples, but no differences were observed between OEP and control radiated samples (OEP 117/1373 8.5% vs control 132/1493 8.8%). Dicentrics were observed only in radiated cells (OEP 14/1373; controls 9/1493), and no rings were observed in any condition. Increased stable aberrations in basal cultures of OEP, evidences chronicle effect damage. Unstable aberrations such as dicentrics in radiated samples, reflect acute radiation effect. Such findings are compatible with an adaptative response of OEP to repair damage efficiently, subsequent to acute radiation stimuli. This procedure was able to detect low dose radiation effect in OEP and may be considered to evaluate laboral safety conditions.

Molecular cytogenetic detection of a (Y;22) translocation in an infertile 45,X male. *S. Brisset¹, A. Aboura¹, M. Misrahi², J.C. Soufir³, V. Izard⁴, R. Frydman⁵, G. Tachdjian¹.* 1) Department of Genetics, Hopital Antoine Beclere, Clamart, France; 2) Department of Molecular Biology, Hopital Bicetre, Le Kremlin Bicetre, France; 3) Department of Andrology, Hopital Bicetre, Le Kremlin Bicetre, France; 4) Department of Urology, Hopital Bicetre, Le Kremlin Bicetre, France; 5) Department of Obstetrics and Gynecology, Hopital Antoine Beclere, Clamart, France.

Previous cases with (Y;autosome) translocations have been reported in association with male infertility. The azoospermic factor (AZF) on long arm of Y chromosome, which is critical for spermatogenesis, may be affected secondary to a microdeletion, rearrangement or complete loss as a result of the translocation mechanism. We describe a new case with a de novo translocation t(Y;22). A 36-year-old male with azoospermia was found to have a 45,X karyotype. Further cytogenetic examination revealed a mosaic 45,X/46,X,+mar. Fluorescence in situ hybridization (FISH) was performed using the following probes: centromeric probes specific for chromosomes X,Y and 14/22, whole chromosome painting probes for chromosome 22 and chromosome Y and DiGeorge locus probe. FISH showed a derivative chromosome Y containing the short arm, the centromere and a small proximal part of the long-arm euchromatin of the Y-chromosome and the long arm of a chromosome 22. The breakpoint on the chromosome 22 occurred in the region just below the centromere. Thus, the small marker included the short arm and the centromere of chromosome 22. This small chromosomal fragment was unstable and lost in the cell line 45,X,der(Y)t(Y;22). Microdeletion analysis of the Y chromosome was performed using STS-PCR approach. Twenty different STS corresponding to the three distinct AZF loci (AZFa, AZFb and AZFc) and to the heterochromatic distal Yq region were amplified. AZFa was present and AZFb and AZFc were deleted. This unbalanced translocation t(Y;22) generated a long arm Y deletion with loss of the AZFb and AZFc regions and Yq heterochromatin region. Our case shows the importance to combine cytogenetic and molecular approaches in the diagnosis of male infertility, especially for genotype-phenotype correlation.

Subtelomeric chromosome abnormalities in South Carolina. *B.R. DuPont, J.S. Collins, R.S. Saul, C.E. Schwartz, R.E. Stevenson.* Greenwood Genetics Ctr, Greenwood, SC.

Fluorescence in situ hybridization (FISH) analysis of chromosome subtelomeric areas has enhanced the search for the underlying etiology in patients with idiopathic mental retardation. Previous studies have shown between 4% and 23% of children with moderate to severe retardation have a subtle chromosome abnormality using subtelomeric FISH probes. The largest study (Knight et al., 1999) found approximately 7% of patients had an unbalanced telomere rearrangement. A subsequent analysis of their data [29 affected, 110 controls] demonstrated that the prenatal onset of growth retardation and a positive family history for mental retardation are good indicators for subtelomeric analysis (STA) (deVries et al., 2001). The Greenwood Genetic Center Cytogenetics Laboratory initiated STA in 2000. To date, we have analyzed 166 people with developmental delay. We found subtelomeric abnormalities in 17 patients (8M, 9F), 14 Caucasian, 1 African-American, 1 Hispanic and 1 Indian. They ranged in age at testing from 2 months to 63 years, with 7 below the age of 9 and 4 above the age of 50; birth weights fell into normal ranges. Clinical features and abnormal test results that occurred in more than one of these individuals included facial dysmorphism (16/17), skeletal abnormalities (4/12), CNS abnormalities (6/9), cardiac abnormalities (6/13), vision abnormalities (4/13), cranial image abnormalities (8/12), and other abnormalities (10/12). The chromosome aberrations included two with 1p-, 2q-, 3q-, 8p-, 8p+, 9p-, 14q-, 15q-, 22q+, t(3;7), der(4)t(4;7), der(4)t(4;11), der(11)t(11;18), der(13)t(5;13), der(14)t(7;14), and der(19)inv(19). Five of these rearrangements were familial in origin, four were de novo, and results on the others were pending. In reviewing the causes of mental retardation in 7600 persons in South Carolina, we found 2513 with moderate to severe MR with unknown causation and an additional 324 with MR and a family history. If these two groups are combined, we might expect a retrospective analysis to find as many as 285 STA abnormalities using the 10% estimate found in our population.

Duplication of 15q24 and panic disorder - Is it real? *S.L. Christian, J. Lyons, F.J. McMahon, E.S. Gershon.* Dept Psychiatry, Univ Chicago, Chicago, IL.

In a recent study three types of dup(15)(q24) were observed in 7 families with panic and/or anxiety disorder comorbid with joint laxity (Gratacos et al. Cell 106:367-379, 2001). Dup(15)(q24q26) and dup(15)(q26q24) involving the telomeric region of the chromosome, as well as, dup(15)(q22q24) were observed in a total of 72% of the affected individuals. In a followup study 67/69 individuals with panic disorder undiagnosed for joint laxity showed one of the three types of dup(15)(q24) for a frequency of 97%. Based on these data we analyzed a series of 10 families collected as part of a National Institute of Mental Health study on the genetic basis of panic disorder for the presence of dup(15)(q24). Cell lines were not available for these initial studies, so microsatellites from the 15q22-q26 region were analyzed to check for either the presence of 3 alleles or a dosage difference between 2 alleles. The distal third of chromosome 15 contains multiple copies of segmental repeated regions, termed LCR15, that are similar to the duplicated genomic segments that rearrange to cause del(15)(q11q13) in Prader-Willi and Angelman syndromes. Therefore, 13 microsatellites including 3 from the reported region of duplication were selected to examine the single copy regions between 10 LCR15s on 15q22-qter. The results of the microsatellite analysis using 3 markers within the reported region of duplication were normal in all 10 families. Additionally, three alleles were not detected for any marker across the entire q22-qter region in any of these families. However, one marker, D15S818, located proximal to the reported region of duplication, displayed possible dosage differences in one allele that may indicate a duplication in this region. To further test the 15q24 region for the presence of duplications, cell lines from an additional 30 patients with panic disorder have been acquired for FISH analysis. In these cases BACs from the single copy regions between the 10 LCRs on 15q22-qter will be analyzed to test for duplications of 15q24.

An unusual de novo derivative/recombinant (14) from a likely germ line rearrangement,t(12;14), contributing to a partial trisomy 14q32.2@14qter. *J. de Oliva Ramirez*¹, *F. Jaquez*¹, *M. Varma*², *S. Kleyman*², *M.J. Macera*², *A. Babu*². 1) Robert Reid Hospital, Santo Domingo, Rep. Dominicana; 2) Div. Mol. Med. and Genet., Dept. of Med., Wyckoff Heights Med. Center, Brooklyn, NY.

A 4 1/2 year old girl was referred for cytogenetic evaluation because of wide fontanelles, macroglosia, preauricular fistula, constipation and non cyanotic congenital cardiopathy. She had mental retardation, with high implantation scarce hair, scarce eyebrows and eyelashes, strabismus, short wide elevated bridge, bulbed nose tip with anteverted nasal nostrils, fine lips, elongated mouth, high and narrow palate. A systolic murmur on the left parasternal border and pectus excavatum. Prominent peri-umbilical tissue, patent umbilical ring, rectus abdominis diathesis and patellar depression. 5th finger clinodactyly, long fingers and skin hyper-elasticity. Cytogenetic analysis of her peripheral blood revealed an abnormal 46,XX,?rec(14)t(12;14)(q21.3;q32.2), with an unusual secondary constriction at what appeared to be the 14q and 12q fusion point. Fluorescence in situ hybridization (FISH) using whole chromosome paints (wcp) for chromosome 12 and 14 confirmed the presence of a chromosome 12 segment in the long arm of der/rec chromosome 14, however, no detectable signal for wcp 14 was seen on the derivative chromosome 12. Telomere specific probe for chromosome 12 q (TelVysion 12q) hybridized to the distal qter of the normal homologue and the der/rec 14. Probe for telomere associated sequences for chromosome 14 (Telvysion 14q) showed signal over the normal 14, no signal over the del(12) but two separate signals in close proximity on the abnormal 14, suggesting a duplication of the 14q32.2 up to and including the telomere associated sequences. This contributes to a partial trisomy of 14 14q32.2@14qter. The karyotype was finally designated as 46,XX,der(12)t(12;14)(q31.3;q32.2).ish ?rec(14) (14pter@14q32.3@14q32.3::14q32.3@14q32.2:12q21.3@12qter)(wcp12+,wcp14+,tel14++). Both of the parents were cytogenetically normal, suggesting a possible multi-step de novo rearrangement. Possible mechanism(s) giving rise to such a complex derivative/recombinant chromosome rearrangement are described.

Subtelomere Deletion of 10q: Greater Frequency in the Population. *I.K. Gadi¹, P.N. Mowrey¹, P. Singh-Kahlon¹, P.R. Papenhausen¹, M. McDonald², P. Jayakar³, C. Bullen¹, J.H. Tepperberg¹.* 1) Dept Cytogenetics, Laboratory Corp of America, Res Triangle Pk, NC; 2) Duke University Medical Center, Durham, NC; 3) Miami Children's Hospital, Miami, FL.

Deletions of distal chromosome 10q appear to be relatively rare with only about 25 cases reported in the literature. We describe three cases with terminal deletions of 10q identified from a total of 1300 FISH subtelomere cases analyzed. Each case was submitted as a follow-up to a previously reported normal karyotype. Case one was a 3-month-old female with microcephaly, cupped ears, sloping forehead, hypertelorism, short stature, PDA, ASD, and abnormal kidneys. Subtelomere and retrospective cytogenetic analysis showed a de novo terminal deletion at 10q26.1. Case two was a 2.5-year-old female with microcephaly, preauricular sinuses, skin tags and bilateral epicanthal folds, broad nasal bridge, smooth philtrum, strabismus and developmental delay. FISH and cytogenetic analysis revealed an extremely subtle terminal de novo deletion at 10q26.3. The third case was a 5-year-old male with microcephaly, hypotonia, short stature, hydronephrosis, strabismus, and global developmental delay. The FISH and cytogenetic analysis revealed a very subtle terminal deletion at 10q26.3. Parental chromosome analyses are pending. Some of the common features reported with apparently larger 10q terminal deletions are growth retardation, psychomotor retardation, triangular face, hypertelorism, cryptorchidism, urinary tract anomalies and hypoplastic kidneys. Our patients appeared to have many of the features reported in the literature suggesting that the genes responsible for the common features may be located distal to 10q26.1. The three subtle 10q subtelomere deletions detected by FISH suggest a hot spot for subtle or cryptic 10q terminal deletions. Such deletions, essentially below the resolution of conventional G-band analysis, confirm the efficacy of subtelomere testing in globally affected patients with apparently normal karyotypes.

Familial MR associated with a subtle duplication in 4p16.3 which includes the WHSCR. *T. Drumheller, R. Vandergon, P. O'Lague, T. Treisman, E. Roeder.* Dept Cytogenetics, Dept Genetic Medicine, Childrens Hospital Central Cal, Madera, CA.

A duplication in distal 4p was identified in a mother and daughter who have mild mental retardation, minor dysmorphic features, tall stature, a normal to large head size and aggressive behavior. Initial chromosome analysis performed on the mother in 1993 reported a normal karyotype. However, a recent study of the daughter revealed a chromosome 4 with extra material at the end of its short arm. Fluorescence *in situ* hybridization (FISH) analysis using a probe for the 4p telomere region showed a normal pattern of hybridization signals. Commercial probes for the area of the Wolf-Hirschhorn critical region (WSCR) consistently showed a brighter signal on one chromosome 4 that could be resolved as 2 signals on elongated metaphase chromosomes. FISH performed on interphase nuclei consistently demonstrated 3 signals for the WHSCR probe. Follow-up studies showed the same duplication in the mother and in a newborn half-sister. The phenotype is mild compared to that described for the 4p Duplication syndrome. The subtle appearance of the duplication underscores the need for careful chromosome analysis at high resolution. A multitelomere screen would not have identified this abnormality. Eagerly anticipated new technologies, such as comparative genomic hybridization (CGH) analysis of micro-arrayed clones, will undoubtedly help identify such interstitial chromosome abnormalities.

Interstitial deletion of 9p in a patient with infertility. *E.H. Cho¹, Y.M. Kim¹, H.M. Ryu^{1,2}, Y.H. Cho³, S.Y. Park¹*. 1) Laboratory of Medical Genetics, Samsung Cheil Hosp, Seoul, Korea; 2) Obstetrics and Gynecology, Samsung Cheil Hosp, Seoul, Korea; 3) Department of Medical Genetics, College of Medicine, Hanyang University, Seoul, Korea.

9p22-p23 was reported as a critical region for the 9p-deletion syndrome that includes dysmorphic facial features and moderate to severe mental retardation. We present a 34 years old woman who referred for facial anomalies with micrognathia, mild mental retardation and infertility. Conventional GTG banded chromosome analysis indicated interstitial deletion in 9p. Subsequent high resolution and reverse banding were interpreted as 46,XX,del(9)(p22.3p23). Further comparative genomic hybridization (CGH) analysis and fluorescence in situ hybridization (FISH) analysis were performed to confirm the interstitial deletion. Results of fluorescence in situ hybridization (FISH) analysis using 9p telomeric probe and whole-chromosome painting probe and specific probe for chromosome 9 were compatible with cytogenetic interpretation. CGH profile revealed lack of chromosomal material at that region.

Prenatally detected triploidy/trisomy 7 mosaic. *M.J. Huggins¹, J.A. Ramsay^{2,3}, J. Xu^{2,3}*. 1) Obstetrics and Gynecology; 2) Pathology and Molecular Medicine; 3) Laboratory Medicine, Hamilton Health Sciences and McMaster University.

We present a rare case of mosaicism of triploidy and trisomy 7 detected prenatally. A 33-year-old woman was referred at 20 weeks gestation because of asymmetric IUGR of a singleton fetus (~17 weeks size) with a probable endocardial cushion defect. Amniocentesis performed following ultrasonography was analyzed by interphase FISH and routine karyotyping. Interphase FISH with Vysis AneuVysion probe set specific to chromosomes 13, 18, 21, X, and Y was performed on uncultured amniocytes. Analysis of 50 interphase nuclei for each probe showed at least 70% of the cells had 3 representative signals indicative of triploidy: nuc ish Xcent(DXZ1x3)[35/50],13q14(RB1x30)[42/50],18cen(D18Z1x3)[38/50],21q22(D21S259x3)[42/50]. Analysis of 21 in situ colonies showed an abnormal female karyotype mosaic for triploidy and trisomy 7: 69,XXX[8, 38%]/47,XX,+7[13, 62%]. The couple requested pregnancy termination. Postmortem examination of this female fetus identified craniofacial dysmorphism, including a conical shaped cranium, small chin, low set ears, and a flattened nasal tip with a prominent nasal groove. There were overlapping fingers on both hands, and bilateral rocker bottom feet with toe syndactyly. An endocardial cushion defect of the heart was confirmed and the left ventricle was hypoplastic. Additional findings included bilateral single lobed lungs, early microcystic changes of the kidneys, hypoplastic ovaries and mild microencephaly. These findings are consistent with triploidy. Follow-up cytogenetic analysis of 70 cultured cells from the fetal tissue showed 69,XXX[60, 86%]/47,XX,+7[10,14%], confirming the prenatal cytogenetics findings. Maternal and paternal karyotypes were normal. To our knowledge, this is the first case report of triploidy/trisomy7. Further DNA investigations may help clarify whether it originates from a single zygote (i.e. mosaicism) or fusion of different zygotes (i.e. chimera).

Deletion 3p25.3 in a mother and daughter with normal phenotype. *G.A. Jervis, P. Newkirk, B.G. Kousseff.* Regional Genetics Program, Univ South Florida, Tampa, FL.

JT, a 36 year old Caucasian woman, G2P1001, had an amniocentesis during her first pregnancy which revealed, 46,XX,del(3)(p25.3). JT was found to have the same terminal deletion. JT had FISH analysis using multiple probes for chromosome 3 to rule out a translocation or inversion. Five metaphase cells were scored using a whole chromosome 3 paint probe (Oncor, Inc) and all cells showed complete hybridization over the entire length, with no other hybridization signals on other chromosomes. Two different sets of subtelomeric probes specific for the short arm of chromosome 3 were hybridized independently to metaphase cells (D3S1443/ D3S1444, Oncor, Inc; D3S4559, Vysis), using a chromosome 3 centromere probe (D3Z1) to identify the chromosome in each hybridization reaction. Ten cells were analyzed with each probe set and all cells demonstrated a deletion of the terminal end of 3p. Skin biopsy revealed the same result without mosaicism. JT's parents had normal karyotypes. Of interest, JT's mentally retarded sister had a de novo unbalanced translocation, 46,XX,add(8).ishder(8)t(4;9)(q31.1-23.2)(wcp4+,wcp+8). JT is a pediatrician, without dysmorphic features. JT's daughter, AT, is a beautiful Eurasian girl, who at 15 months of age was developing normally with weight at 25th centile; height at 30th centile and head circumference at 50th centile. With her current pregnancy, JT is having CVS due to the 50% risk for her fetus to have 3p deletion. The phenotypic expression of this deletion may vary due to genomic imprinting and may result in physical consequences. Without genomic imprinting, the deleted segment may be genetically inert and of no clinical significance. Terminal deletions of 3p have been reported and most involved loss of the 3p25 band. The most common features included low birth weight (70%), severe postnatal growth retardation (100%), severe mental retardation (100%), microcephaly (80%), brachycephaly, and unusual facies (Gorlin, 2001). We present a mother and daughter with 3p25.3 deletion and apparently normal phenotype. This suggests that the critical region for the phenotype of 3p deletion syndrome lies within the region 3p25 to 3p25.3.

Partial trisomy 22 resulting from an extra chromosome 22 with an interstitial deletion. *A.S. Kulharya, S.M. Delaney, D.B. Flannery.* Dept Pediatrics, Medical Col Georgia, Augusta, GA.

Partial trisomies for the distal region of chromosome 22 have been described as unbalanced products resulting from segregation of a reciprocal translocation with another chromosome. Most markers derived from chromosome 22 are either dicentric, with two copies of the proximal region that result in Cat Eye syndrome, or they are products of 3:1 nondisjunction of t(11;22) resulting in der(22) syndrome with partial trisomy for distal 22, both with characteristic phenotype. We present a patient with partial trisomy 22 for the distal region resulting from an extra chromosome 22 with an interstitial deletion of the 22q11.2 region. Since the 22q11.2 region is prone to various kinds of rearrangements at a higher frequency than any other chromosome region it is surprising that such marker chromosomes have not been reported previously. The patient was evaluated at one day of age and has been followed for the past one year. She has a broad forehead, almond shaped eyes, cleft palate, short neck with redundant skin, wide spaced nipples, posteriorly rotated low set ears and metatarsus adductus. The nails on the halluces are very hypoplastic. A pneumothorax was treated with chest tubes. Chromosome analysis demonstrated an extra satellited marker chromosome in all the metaphases analyzed. The G-banding and FISH analysis with various probes revealed the karyotype to be: 47,XX,+der(22)(pter->q11.1::q13.1->q13.1->qter). The probe D22S75 that hybridizes to the 22q11.2 region was deleted in the additional chromosome. However, a signal for the probe specific for the ARSA gene in 22q13.1 region was observed on this chromosome. The parental karyotypes were normal. Our patient has few features of complete trisomy 22, such as webbed short neck and cleft palate which have been observed in patients with duplications of the distal 22q. However, the only feature common among our patient and patients with der(22) syndrome is cleft palate, which is unusual as both have partial trisomy of the same region of chromosome 22. This case provides a genotype phenotype correlation in case of over expression of genes in distal region of chromosome 22 without the involvement of another chromosome.

SEGREGATION OF A t(1;3) TRANSLOCATION IN MULTIPLE AFFECTED FAMILY MEMBERS WITH BOTH TYPES OF ADJACENT-1 SEGREGANTS. *C. Kozma¹, A. Slavotinek², J. Meck¹*. 1) Department of Pediatrics/Departemnt of Obstetrics and Gynecology, Georgetown University, Washington,DC; 2) National Human Genome Research Institute, National Institute of Health, Bethesda, MD.

A subtle familial balanced translocation involving the terminal regions of 1q and 3p was identified in a large family by high-resolution karyotype analysis and confirmed by fluorescence in situ hybridization (FISH) analysis. In this family, segregation of a balanced t(1:3)(q42.3;p25) chromosome translocation in two phenotypically normal sisters led to two types of viable unbalanced complements. The proband inherited the derivative chromosome 3 resulting in partial trisomy of 1q and partial monosomy of 3p. A paternal uncle and cousin had the reciprocal rearrangement with a derivative of chromosome 1 resulting in partial monosomy for 1q and partial trisomy for 3p. While profound mental and physical retardation and congenital heart defects were characteristic for both rearrangements, facial dysmorphism was quite distinct for each imbalance. Individuals who had the derivative chromosome 3 had a long face, wide eyebrows, small palpebral fissures, hypertelorism, prominent glabella, a large tip of the nose, long philtrum with thin upper lip, and low set-ears. In contrast, family members with the derivative of chromosome 1 had a tall forehead with bifrontal narrowing, full and large cheeks, and large simple ears. Since the translocated segments are small and approximately equal in size in this family, it is not surprising that viability was seen in individuals with both types of adjacent-1 segregation. In this kindred, the ratio of normal to abnormal individuals born to balanced carriers is believed to be 1:1.5. This suggests that the recurrence risk for carriers is at least as great as 50%.

Complex chromosome rearrangement in a case of assisted reproduction by IVF-ICSI. *C. Lostau*¹, *M. Orera*¹, *P. Blanco*², *C. Puertas*², *M.D. Lillo*¹, *C. Alonso*³. 1) Genetics, H. Gregorio Maranon, Madrid, Spain; 2) Biochemistry, H. Gregorio Maranon; 3) Clinical Analysis. H. de la Princesa.

INTRODUCTION: The widespread use of assisted reproduction has raised questions about the potential risks of the procedures and the effect in the fitness of the offspring. Intracytoplasmic sperm injection has been specially controversial given the lack of sperm selection. **MATERIAL AND METHODS:** 36 years old pregnant woman that underwent in vitro fertilization with intracytoplasmic sperm injection. Both the ovum and sperm were obtained from unknown donors. The husband is azoospermic with a 46 XY karyotype. She has regular cycles and her karyotype is 46 XX. The patient comes to our Genetic Unit, because of an elevated risk for Downs syndrome. The karyotype of the amniocytes revealed a single cell line with a complex rearrangement involving chromosomes 4,7 and 9 that was described as 46 XX, rcp (4;7) (q21;p15) rcp (7;9)(q23;p22). We confirmed the same karyotype in cord blood. We were able to obtain clinical data of the ovum donor, including the karyotype that was normal. The sperm sample had been purchased by the IVF clinic in a private sperm bank that was unable to identify the donor nor to provide data with the exception of a normal sperm count. We informed the patient that the phenotype of the fetus was unpredictable. We designed a follow up program that included sequential ultrasounds up to the 26th gestational week and a Doppler assisted ultrasound in the 22nd week. Providing that all the results were normal, the patient decided to continue the pregnancy. In the 38th gestational week she delivered a phenotypically normal girl, with APGAR 7-9, that weighted 2.800 gm. The girl is 14 months old and her development is within normal limits. **DISCUSSION:** The case illustrates most of the difficulties that can be encountered in the management of gestations attained by assisted reproduction. The impossibility to identify the sperm donor, rendered the phenotype prediction unattainable. Finally, it is important to emphasize the fact that the decision to continue the pregnancy was based in the apparent cytogenetic balance and the absence of ultrasound anomalies.

Chromosomal abnormalities in epithelial and blood cells of children with two types of Inflammatory Bowel Disease. *N.T. Holland¹, P. Harmatz², Y. Wu¹, J. Bae¹, R. Momi¹, D. Golden¹, J. Cooper³, J. Madden², C. Chen¹, A. Hubbard¹, M. Heyman³.* 1) Division of Environmental Health Sciences, University of California, Berkeley, Berkeley, CA; 2) Children's Hospital Oakland Research Institute, Oakland, CA; 3) Department of Pediatric Gastroenterology, Hepatology and Nutrition, University of California, San Francisco, CA.

Inflammatory Bowel Disease (IBD) includes two primary diagnoses: Crohn's disease and ulcerative colitis (UC). Both types of IBD are characterized by inflammatory infiltrates in the gastrointestinal tract, folate deficiency and an increased risk of cancer. Though genetic predisposition is suggested, the pathological mechanism remains unknown. The goal of this prospective case control study involves a longitudinal assessment of biomarkers of genetic damage in 31 IBD patients (19 CD and 12 UC) and 23 controls. The objective is to establish whether IBD in children is associated with an increased level of cytogenetic abnormalities, and whether it can be improved by folate supplementation. IBD subjects and controls were matched for age (12 years) and sex (56% males). Blood, urine and buccal samples were collected twice, first at the time of initial diagnosis and after one month of treatment and folate supplementation (IBD patients) or only folate supplementation (controls). Cytogenetic damage was assessed by micronucleus (MN) assay. Genetic endpoints in human lymphocytes included frequencies of MN and bridges. Cell death was assessed by percentage of apoptotic and necrotic cells and cell proliferation was measured by replicative index. CD and UC patients were different for most of the endpoints and they had a higher level of damage than controls. Treatment and folate supplementation had a positive effect on the levels of cell death, and decreased cytogenetic damage in IBD patients. Control subjects benefited from folate supplementation as indicated by a decrease of cytogenetic damage in their lymphocytes and epithelial cells. Supported by grants from Lucille Packard Foundation, Crohn's and Colitis Foundation of America and NIH (M01 RR01271).

A complex chromosomal rearrangement in a girl with mental retardation and distinctive dysmorphic features.

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We report a 4 year old female with a de novo complex chromosomal rearrangement and a distinctive phenotype. Her medical history is significant for having been a twin at 35 weeks gestation, breech presentation, with feeding problems and poor growth as an infant, gastroesophageal reflux disease, peripheral pulmonic stenosis, omphalocele, high myopia and severe mental retardation. She is small for her age with microcephaly, posteriorly sloping forehead, shallow orbits, long palpebral fissures, prominent nose, wide mouth, absent uvula, along with kyphosis, brachydactyly, bridged palmar crease and hypertonia. Peripheral blood lymphocytes revealed a karyotype of 46,XX,t(1;12)(p22.3;q21.3),inv(6)(p24q23),t(7;18)(q11.2;q21.2) in all cells. Parental chromosomes and those on her twin were normal. Spectral Karyotyping (SKY) and FISH with whole chromosome paints for chromosomes 1, 6, 7, 12, and 18 did not reveal additional chromosomal rearrangements. However, prometaphase G-banding analysis suggests the inverted chromosome 6 may contain a cryptic duplication, deletion, or other rearrangement which might be causative for the observed phenotype. Additional studies are under way to elucidate the nature of the abnormality.

Comparison of three cases of deletion and duplication in 5p. *K.M. May, D. Saxe, A. Yearell, C. Taylor, K. Uhas.*
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The frequency with which structural abnormalities occur in any given chromosomal location may be directly related to the positions of repetitive DNA sequences within the region. The short arm of chromosome 5 has been described as one area that is prone to rearrangement on that basis. We present three unusual and unrelated cases of live born individuals with deletion and duplication of 5p. As is the case with most chromosome rearrangements which contain inverted duplications, all three cases also have terminal deletions. The first case has a deletion of p15.31 and duplication of p13.3 to p15.31. The second case has a terminal deletion at p15.1 and a duplication of p12 to p15.1. The third individual has two cell lines with different structural abnormalities of chromosome 5. One cell line contains a terminal deletion at p15.1 without duplication, and the other line has a deletion at p15.31 and an inverted duplication of p12 to p15.31. These three cases have both monosomy and trisomy for varying amounts of 5p. The three cases have been characterized with FISH probes in the 5p region to more accurately determine the extent of imbalance. We compare the phenotypes with features associated with deletions and duplications of 5p, and as expected, there is overlap in each case with both monosomy and trisomy.

1p36 deletion syndrome: Refinement of a 2 Mb critical region. *K. Kurosawa*¹, *H. Kawame*², *Y. Ochiai*³, *A. Akatsuka*³, *M. Kobayashi*³, *N. Okamoto*⁴, *K. Imaizumi*¹, *Y. Kuroki*¹. 1) Kanagawa Children's Med Ctr, Yokohama, Japan; 2) Nagano Children's Hospital, Nagano, Japan; 3) Tokyo Metropolitan Kita Med Ctr, Tokyo, Japan; 4) Osaka Maternal & Children's Med Ctr, Osaka, Japan.

1p36 deletion syndrome is a newly delineated MCA/MR syndrome characterized by mental retardation, growth delay, epilepsy, congenital heart defects, characteristic facial appearance, and precocious puberty. We here report on 7 cases with the disorder identified by FISH screening with D1Z2 among MCA/MR. All the cases had normal karyotype of standard GTG banding. All have severe to profound mental retardation. Feeding difficulty is common to the cases. Five of 7 have intractable epilepsy and 2 are free from seizures. Two of 7 have dilated cardiomyopathy. To define the chromosomal deletion at the molecular level in the 1p36 deletion syndrome, we analyze 7 cases by FISH with BACPAC genomic clones in the 1p36 region. The common deletion is about 2 Mb between RP11-304C7(D1S3672) and RP4-785P20(NBR). The severity of clinical symptoms is correlated with the deletion size, demonstrated by two cases with profound mental retardation who have 9 Mb deletion. Haploinsufficiency of potassium channel beta-subunit (KCNAB2) are thought to be responsible for the intractable seizures found in the disorder, but it is not the case for our 3 of 7 cases. 1p36 deletion syndrome is one of the most frequent terminal deletion syndrome, account for 0.5-0.7% of MR. All our cases were recognized as 1p36 deletion by clinical features, and confirmed by FISH with D1Z2. Refinements of critical regions is important for the search for the causative genes for the syndrome.

Ring 2 chromosome: structural analysis by FISH. *T. Kosho*¹, *K. Matsushima*², *T. Sahashi*³, *R. Muto*⁴, *N. Mitsui*², *H. Sogajima*³, *H. Ohashi*⁵. 1) Department of Pediatrics, Saitama Municipal Hospital, Saitama, Saitama, Japan; 2) Department of Clinical Laboratory, Saitama Children's Medical Center, Iwatsuki, Saitama, Japan; 3) Department of Pediatrics, Nagoya Daini Red Cross Hospital, Nagoya, Aichi, Japan; 4) Department of Pediatrics, Tokyo Women's Medical University, Shinjuku, Tokyo, Japan; 5) Division of Medical Genetics, Saitama Children's Medical Center, Iwatsuki, Saitama, Japan.

We report a female of ring 2 chromosome with fine structural analysis by FISH. She was born after 33 weeks' pregnancy, which was complicated by intrauterine growth retardation and oligohydramnios. Birth weight was 1,276 g (-1.7 SD), length 38.0 cm (-2.3 SD), and head circumference (OFC) 26.0 cm (-2.1 SD). She exhibited respiratory distress syndrome, small ventricular septum defect (VSD), and hyperbilirubinemia in the neonatal period. She was fed through nasogastric tube until six months of age, because of poor sucking. At 19 months of age, she demonstrated severe proportionate growth retardation (weight 5,476 g (-5.3 SD), height 66.5 cm (-4.8 SD), and OFC 39.5 cm (-5.0 SD)) but her motor and mental development was normal. Her clinical features included mild hypertelorism, low and broad nasal root, thick upper and lower lips, flat occiput, and clinodactyly of bilateral fifth fingers. VSD was closed spontaneously. She suffered from frequent lower respiratory infections and eczema. Chromosome analysis using peripheral blood lymphocytes revealed 46,XX,r(2)(p25.3q37.3) constitution in 66/100 cells studied. Mitotic instability of the ring was observed in the other cells. FISH analysis using chromosome-specific subtelomeric probes (2ptel, 2qtel) showed no loss of sequences specific for subtelomeric regions of 2p and 2q. This is the ninth case of ring 2 chromosome and the first report in which fine structural analysis by FISH was performed. The breakpoints at 2pter and 2qter existed distal to chromosome-specific subtelomeric probes. The etiology of severe growth retardation without major malformations may be related not to loss of chromosomal materials but to instability of ring chromosome.

Frequent rearrangements between low copy repeats on 22q11 (LCR22) and telomeric bands of translocation partner chromosomes. *B.E. Morrow¹, E. Spiteri¹, K. Wakui², V. Pulijaal³, S. Minoshima⁴, N. Shimizu⁴, L.G. Shaffer².*
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The chromosome 22q11 region is susceptible to rearrangements resulting in three different congenital anomaly disorders, velo-cardio-facial syndrome/DiGeorge syndrome (VCFS/DGS), der(22) syndrome and cat-eye syndrome (CES). Meiotic homologous recombination events between two blocks of low copy repeats on chromosome 22 (LCR22s) mediate recurrent rearrangements associated with VCFS/DGS and CES. The translocation in carriers of the constitutional t(11;22) translocation is mediated by double strand breaks in AT-rich palindromes on both chromosomes 11q23 and 22q11, within a third LCR22. Malsegregation of this translocation leads to der(22) syndrome. Therefore, both homologous and non-homologous recombination events between LCR22s mediate recurrent rearrangements on 22q11. In addition to these recurrent rearrangements, non-recurrent, unique rearrangements on 22q11 have been identified, in particular, reciprocal translocations. We analyzed cell lines derived from fourteen different individuals with non-recurrent reciprocal translocations by genotype analysis, FISH mapping and somatic hybrid studies to determine whether there are common features among them. We found that five occurred in the same LCR22 as for the t(11;22) translocation implicating the same AT-rich palindromes. In addition to these cases, four others had chromosome breakpoints in the vicinity of different LCR22s, while five were in non-LCR22 sequences. The breakpoints on the partner chromosomes were all located in their most telomeric band, half in the unique regions and half in the vicinity of distal telomere associated repeats (TARs). Therefore, most of the translocation breakpoints occurred in highly dynamic regions of the genome, 22q11 and telomeric bands. Understanding the molecular basis for susceptibility to rearrangements on 22q11 can serve as a model for genomic disorders on other chromosomes as well.

Clinical features associated with an add(18)(q21) representing partial duplication of chromosome 18. K.J.

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A 44 year old caucasian male was seen for genetic assessment of developmental disability. Birth history was of a 10 day post-term induced vaginal delivery with low forceps use but no reported injury. Bilateral club feet and pes cavus were identified at birth. As an infant he was hard to feed and had frequent constipation. Additional features included bilateral atresia of the ear canals, left moderate mixed hearing loss, left fibular hypertrophy, urachal cyst and anomalous 1st and 2nd ribs. Medical problems have included recurrent otitis media, pneumonia, asteatotic exzema, dshydrosis, chronic paralytic ileus, epilepsy and factor VIII hemophilia. On examination, growth parameters were normal. He had a wide mid-face with prominent cheeks; thin, pointed nose with an upturned tip; a flat, short philtrum; thin upper lip; dental malocclusion; abnormal ear shape; wide-spaced nipples; long sternum; narrow hands and brachydactyly. He had a high-stepping, ataxic gait. G-banded chromosome analysis identified a de novo 46,XY,add(18)(q21) karyotype with an extra G-positive band at the terminal end of the chromosome. Fluorescence in situ hybridization (FISH) studies using a whole chromosome paint probe specific for chromosome 18 identified the add(18)(q21) to be entirely chromosome 18 derived. The nature of the chromosome rearrangement could not be discerned by G-banding analysis. The extra G-positive band could represent a duplication of 18q12.1 or 18q22.1 material, although it is most likely part of a complex rearrangement. Molecular cytogenetic analysis using comparative genomic hybridization will provide additional information concerning the genetic imbalance involved with this rare chromosome 18 rearrangement.

Characterization of supernumerary marker chromosomes of chromosome 22 origin and associated with normal phenotype. *H.E. McDermid¹, N. Spinner², S. Timshel³, C.H. Gravholt⁴, U. Friedrich⁴.* 1) Dept Biological Sci, Univ Alberta, Edmonton, AB, Canada; 2) Div of Human Genet & Mol Biol, Children's Hosp Philadelphia, Philadelphia, Pa; 3) JF Kennedy Institute, DK-2600 Glostrup, Denmark; 4) Aarhus Kommunehospital/Aarhus University, 8000 Aarhus C, Denmark.

Supernumerary marker chromosomes (SMC) can be associated with both normal and abnormal phenotype, presumably dependent upon the amount and region of the genome in excess. In particular, small SMCs of chromosome 22 origin can be associated with the cat eye syndrome, characterized by defects of the heart, eyes, anus, kidneys, face and mental retardation. This creates a dilemma when an SMC is found prenatally, since its size is not necessarily indicative of whether it will be associated with an abnormal phenotype. We have therefore used FISH to characterize the SMCs of 4 apparently normal individuals and one fetus of unknown phenotype. Each SMC had previously been identified as originating from either chromosomes 14 or 22. These SMCs varied considerably in size, but all were smaller than a G-group chromosome. With chromosome 22-specific centromeric probes D22Z2 and D22Z4, FISH analysis showed that 4/5 SMCs were of chromosome 22 origin. However, because there was significant variation in the strength of the signal of both probes, including a normal chromosome 22 which was negative for both probes, the absence of signal from D22Z2 and D22Z4 cannot be taken as definitive proof of chromosome 14 origin of the remaining SMC. For the 4 SMCs with confirmed chromosome 22 origin, we performed FISH using the cosmid probes N89E2 (closest known unique probe to the centromere) and N107D6 (located in the 1.5 Mb pericentromeric region of 22q11). All four SMCs were negative for both probes, indicating that they contain only centromeric DNA. Therefore, this FISH analysis allows one to distinguish between SMCs which contain only centromeric DNA and are associated with normal phenotype, and previously characterized SMCs which contain at least the first 3 Mb of 22q11 and are associated with cat eye syndrome.

Mosaicism for two different Robertsonian translocations associated with infertility. *L. Martelli¹, D.B. Dentillo¹, J.F. Cuzzi¹, S.A. Santos¹, R.J. Ferreira¹, A.C.C. Nassr², V.M. Motta², E.S. Ramos¹.* 1) Dept Genetics, School of Medicine of Ribeirao Preto - University of Sao Paulo, Brazil; 2) Division of Genetics, Sinha Junqueira Maternity, Ribeirao Preto, SP, Brazil.

The great majority of Robertsonian translocations involve nonhomologous chromosomes and the 13;14 translocation is considered the most common single rearrangement in human beings. Those involving the fusion of homologs are rare. There is an excess of Robertsonian heterozygotes among couples who present recurrent spontaneous abortions. We reported a young, healthy nonconsanguineous couple referred for genetic counseling because of four miscarriages between 8th and 10th weeks gestation. The family history was negative for congenital anomalies. Cytogenetic studies performed on peripheral blood lymphocytes using GTG, CBG, NOR and high resolution bandings revealed a mosaicism for two different translocations: 45,XY,der(13;14)(q10;q10) in 54% of cells and 45,XY,der(13;13)(q10;q10) in 46% of metaphases, without normal diploid clone. The wife's karyotype was normal. Double-colour FISH technique using 13q plus 14q painting probes confirmed the diagnosis. According to the literature, the population frequency for the t(13;13) is 3% and for the 13;14 translocation is around 33%. In the nonhomologous translocation, such as rob(13q14q), there are two options for obtaining gametes: the alternate segregation, which produces normal and balanced gametes and the adjacent segregation producing two types of disomic and two types of nullisomic gametes. Homologous Robertsonian translocation leads to generation of only nullisomic and disomic gametes, essentially a 1:0 segregation, and no normal conceptuses can be produced. We did not find any previous description in the literature about mosaicism for two translocations involving two different chromosomes in somatic cells. In this case, the cytogenetic investigation was essential for the genetic counseling, resulting in indication for preimplantation genetics diagnosis. Supported by FAEPA-HCFMRP, University of Sao Paulo.

Developmental Genome Anatomy Project (DGAP): Mapping Genes That Cause Congenital Anomalies. S.R.

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DGAP was established to identify developmentally important genes by analyzing samples from patients with balanced chromosomal rearrangements and at least one major congenital anomaly. We hypothesize that in a subset of these patients a gene critical in human development will be disrupted or dysregulated by the breakpoint and cause the abnormal phenotype. DGAP consists of four phases: collection of patient samples and detailed clinical histories; FISH-mapping of the breakpoints using BACs at ~1 Mb intervals; sequence analysis and candidate gene identification in the breakpoint regions; and functional analysis of candidate genes in model organisms (mouse and *Drosophila*). We have formed a growing international network of collaborators among clinical cytogeneticists, clinical geneticists and genetic counselors and now have over 100 cases in our database from patients with a variety of developmental defects and apparently balanced rearrangements. We have FISH-mapped 45 breakpoints using a collection of BACs with ~1 Mb resolution. Eleven of these have been localized within single clones, and FISH with both overlapping clones and PCR products has further narrowed the regions of interest. In six cases candidate genes have been identified and are now undergoing detailed molecular analyses. Eight additional breakpoints have been flanked by closely spaced BACs and are also being assessed molecularly. Cytogenetic, clinical, and FISH data for each case can be accessed at our website, <http://dgap.harvard.edu>. As DGAP evolves, and additional breakpoints and candidate genes are identified, we believe it will be a powerful resource for scientists in the genetics and developmental biology communities.

Prenatal diagnosis of a trisomy 21 fetus with an unusual familial t(15;21) translocation. *K.C. Kim¹, K.E. Jackson^{1,2}, G. Pridjian^{1,2,3}, R. Mao⁴, M.M. Li^{1,2}.* 1) Hayward Genetics Center; 2) Department of Pediatrics; 3) Department of OB-GYN, Tulane University School of Medicine, New Orleans, LA; 4) DNA Diagnostic Laboratory, University of Utah, Salt Lake City, UT.

A pregnant woman at 14 weeks gestation was referred for prenatal diagnosis because of advanced maternal age. Cytogenetic studies revealed the presence of an extra chromosome 21 and a satellited marker chromosome reported as 48,XX,+21,+mar. The pregnancy was terminated a week later and the result was confirmed in the fetal tissues. Parental chromosomal studies found that the father carried an unusual 15/21 translocation and a minute chromosome described as 46,XY,t(15;21)(q11.2;p13). FISH using probes D15Z1 and SNRPN showed that the minute chromosome [der(15)] was positive for the D15Z1 and negative for the SNRPN while the der(21) was positive for the SNRPN and negative for the D15Z1. These results confirmed that the t(15;21) breakpoints were on 15q11.2 and 21p13, unlike the traditional robertsonian translocations. FISH studies on the fetus demonstrated that the marker was in fact the der(15) inherited from the father. This extra copy of the chromosome 15 centromere region would have no significant phenotypic consequences since the marker did not contain the PWS/AS region. Genetic counseling uncovered that two paternal uncles of the fetus were diagnosed with Down syndrome. However, they are not available at present time for study. To determine the parental origin of the extra chromosome 21, polymorphic DNA markers on chromosome 21 were studied. Two markers were informative; the parents were heterozygous for both markers, while the fetus was homozygous for both markers. This ruled out meiosis I nondisjunction from either parent. Therefore, the extra 21 must have arisen from either meiosis II nondisjunction or early stage postzygotic nondisjunction. In addition, the balanced translocation may have played a role in the development of the trisomy 21 based on the family history of multiple Down syndrome patients. Future studies on the maternal uncles may shed more light on determining if and how the balanced translocation might have been involved in the chromosome 21 nondisjunction in the fetus.

FISH characterization of a rec(13) in a child with mild MR and his half-sibling with two structurally rearranged chromosomes 13. *S. Mehra, L. Christ, C. Curtis, S. Schwartz.* Center for Human Genetics and Department of Genetics, Case Western Reserve University and University Hospitals of Cleveland, Cleveland, OH.

Pericentric inversions of chromosome 13 are rare with most cases ascertained either through fetal loss or congenital anomalies. In general, the frequency of inversions is estimated to be 1.4 per 1000 varying between 0.3 to 5.0 per 1000 cases. In this study, we describe a six-year old child with mild mental retardation, developmental delay and learning disabilities. He was found to be FRAXA negative with a male karyotype but with a rearrangement of chromosome 13, which appeared to include a duplication of 13q31-qter and a deletion of 13p12-pter regions. The origin of the additional material was confirmed by FISH using a painting probe for chromosome 13. Further studies revealed that his father had a normal karyotype but his mother carried a balanced inversion of chromosome 13. FISH with an acrocentric beta satellite probe showed a hybridization pattern, confirming the rearrangement was an inversion rather than a shift of 13q31-qter into 13p12 and involved a break at p12 within the short arm in the mother. Thus the recombinant chromosome 13 [rec(13)dup(13q)inv(13)(p13q31)mat] in the child arose as a result of meiotic recombination between the normal 13 and inv(13) in the mother. It is likely that the phenotypic abnormalities in the patient derive from imbalance in 13q31-qter. The proband has a half-sibling who was unexpectedly found to have two chromosome 13 structural rearrangements; specifically a balanced inversion similar to his mother's and a balanced translocation, t(13;15)(q12.1;q13) inherited from his father. Thus in both chromosomes, the segment 13p12-13q12.1 is involved and could be expected to disrupt meiotic pairing. This is an interesting and instructive case for several reasons including: (1) The presence of a recombinant chromosome resulting from a maternal inversion; (2) A maternal inversion could be differentiated from a shift of chromosomal material by the use of a satellite DNA probe; and (3) The fortuitous finding of two rearrangements in the half-sib, which has serious implications for pairing and possible future fertility problems in this individual.

A familial interstitial duplication of 22q11.2 with distinct craniofacial and digital anomalies. *S.J. Hased, D. Hopcus-Niccum, L. Zhang, S. Li, J.J. Mulvihill.* Dept Pediatrics/Genetics, Univ Oklahoma Medical Ctr, Oklahoma City, OK.

Refined FISH analysis can reveal undetected chromosomal rearrangements. A patient was referred with cleft palate, hydronephrosis, and minor dysmorphic features, including low set, posteriorly rotated ears, down-slanting palpebral fissures, mandibular micrognathia, and brachymesophalangia. Routine chromosome analysis identified no abnormality of chromosome 22; FISH analysis with the TUPLE1 probe disclosed an interstitial duplication of 22q11.2. Family history showed two older full siblings: a brother with asthma, allergies, ADHD, oppositional defiant disorder, and learning problems and a sister with hydronephrosis and mild delays. Both siblings had similar facial features and were said to resemble the father and several members of his family. Indeed, the father had the same distinctive facial features; he had some post high school education and was in the armed services. He had one full brother and one full sister with developmental delay. Two female first cousins of the father also had hydronephrosis. One half-sister of the paternal grandfather had a cleft palate and died of a respiratory infection at three months of age. The patient's parents had no miscarriages. Subsequently, both older siblings of the proband and their father were found to have interstitial duplications of the TUPLE1 probe. On the initial testing, FISH analysis of metaphase chromosomes did not reveal the duplication, although, on review, the area appears brighter on one chromosome in each metaphase spread. However, FISH analysis of interphase spreads clearly showed three TUPLE1 probe sites with two chromosome specific identification probes in each interphase cell. This family illustrates the utility and need for careful analysis of interphase cells even in samples where good quality metaphases are available and may represent a novel craniofacial syndrome. *TUPLE1.*

Recurrent Miller-Dieker syndrome caused by paternal transmission of a de novo cryptic reciprocal translocation involving chromosomes 16 and 17. *K. Krabchi¹, M. Bronsard¹, R. Laframboise², R. Drouin¹.* 1) Dept. of Medical Biology, St Francois d'Assise Hospital, Laval University, Quebec, Canada; 2) Dept. of Pediatrics, Service of medical genetics, CHUQ, CHUL, Quebec, Canada.

We report non-consanguineous parents with three conceptions with Miller-Dieker syndrome (MDS) out of six pregnancies. The first conception resulted in the perinatal death of a baby with pathognomonic clinical manifestations of MDS. The second and fifth pregnancies ended with a spontaneous abortion. A clinically normal daughter was born in the third pregnancy. Our investigations focused on the proband, who was the fourth conception. This pregnancy resulted in the perinatal death of a baby girl born at full term but with clinical and neuropathological features of MDS. The mid-trimester ultrasound evaluation had shown a slight intrauterine growth delay, polyhydramnios and multiple heart defects. After birth, brain malformations (lissencephaly) were detected by magnetic resonance imaging. GTG-banding studies performed on amniotic fluid cells of the proband were normal. High-resolution (more than 550 bands per haploid genome) GTG-banding analysis of the chromosomes of the parents was normal. Fluorescence in situ hybridization (FISH) using D17S379 marker locus probe specific to MDS/LIS1 was performed on the fibroblasts of the proband and showed a microdeletion affecting the band 17p13.3. We used specific subtelomeric probes to show a de novo paternal cryptic translocation $t(16;17)(q24.3;p13.3)$ that gave rise to the 3:1 segregation unbalanced translocations detected in the conceptions. The sixth pregnancy was interrupted after the same unbalanced karyotype was detected by amniocentesis. The affected conceptions inherited a derivative chromosome 17 with trisomy for 16q24.3 to 16qter, and monosomy for 17p13.3 to 17pter. Facial, skeletal and brain anomalies (agyria) consistent with MDS were found. This pedigree underlines the importance of considering cryptic chromosome anomalies when more than one child presents with congenital anomalies and dysmorphisms. The identification of such cryptic anomalies is critical for informed genetic counseling.

Paternal balanced translocation, t (1p: 19q)(p32; q13.4) associated with recurrent fetal loss - A case report. *D.S. KRISHNAMURTHY, N. AL-TORKI, L. BASTAKI, R.I. AL-BALLOL, S.A. AL-AWADI.* CYTOGENETICS, KUWAIT MEDICAL GENETICS CENTER, SULAIBIKAT P.O., KUWAIT.

Balanced/Unbalanced structural aberrations (deletions/translocations) involving short arm of chromosome 1(1p) are very rare. Unbalanced structural rearrangements (duplications/deletion) are often incompatible with fetal survival and are associated with an increased risk of prenatal loss. Healthy consanguineous couple (First cousins) were referred for cytogenetic investigations because of 5 consecutive abortions. Karyotype of the wife showed normal 46,XX. A balanced translocation, t (1p:19q)(p32: q13.4) was observed in the husband. The couple have two normal male and a female sibs. The family history is unremarkable. The fact that there is no living child associated with deletion/duplication of 1p implies that partial trisomy 1p could be lethal or can result in early fetal loss/stillbirth. The effect of this rare translocation will be discussed in light of other cases reported in the literature involving chromosome 1p and other autosomes/sex chromosomes.

Breakpoint junction fragments in Prader-Willi and Angelman syndrome (PWS/AS) deletion patients reveal variable breakpoints within large duplicons. *S.K. Mewborn¹, N.L. Miley¹, J.A. Fantes^{1,2}, R.L. Brown¹, M.G. Butler³, S.L. Christian⁴, D.H. Ledbetter¹.* 1) Dept of Human Genetics, Univ of Chicago, Chicago, IL; 2) MRC Human Genetics Unit, Western General Hospital, Edinburgh; 3) Children's Mercy Hospitals, Univ of Missouri-Kansas City School of Medicine, Kansas City, MO; 4) Dept of Psychiatry, Univ of Chicago, Chicago, IL.

Genomic duplicons have been implicated at the breakpoints of many contiguous gene deletion syndromes. The most characterized are those mediating Charcot-Marie-Tooth disease type 1a (CMT1A) or hereditary neuropathy with liability to pressure palsies (HNPP) duplications and deletions. Duplicated sequences of 24 kb map to the breakpoints in direct orientation, implicating homologous recombination with a single hotspot within the duplicons as the mechanism. A similar mechanism has also been implicated in deletions of proximal 17p resulting in Smith-Magenis syndrome (SMS). In contrast, large duplicons (450 kb) with >98% homology have been mapped to the breakpoints in PWS/AS patients and appear inverted in orientation. Pulsed field gel electrophoresis (PFGE) analysis has been performed to screen 24 PWS/AS patients for junction fragments. Using three enzymes and seven probes, we have detected seven junction fragments in our patients. These junction fragments are variable in size indicating that breakpoints are not identical across patients as seen in CMT1A and SMS. Large duplicons flanking the Williams-Beuren syndrome (WBS) deletion region at 7q11 also appear to be largely inverted in orientation. Recently a polymorphic inversion of the WBS region has been identified in parents of some deletion patients. We have identified three PWS/AS patients with apparently identical junction fragments. These three patients may indicate the presence of a particular hotspot within the duplicon or a predisposing factor such as an inversion in the parents. These results indicate that a hotspot or precise breakpoint is responsible for the deletions of some patients, while a more random distribution of breakpoints throughout the duplicon may be responsible for the more variable junction fragments seen in other patients.

Cryptic subtelomeric rearrangements: report of two new cases. *E. Lemyre*^{1,2}, *J. Michaud*^{1,2}, *N. Lemieux*³, *F. Tihy*¹.
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Cryptic subtelomeric rearrangements explain a significant proportion of cases with mental retardation. FISH with probes specific to the subtelomeric regions from each chromosome arm was performed on patients with mental retardation and dysmorphic features. We present 2 interesting cases where unbalanced subtelomeric rearrangements were found. The first case is an 18-year-old boy with severe mental retardation associated with metopic craniosynostosis, cleft palate, ASD, pre-axial polydactyly, arachnodactyly, scoliosis, deafness and facial dysmorphism. FISH for subtelomeric regions revealed the unbalanced product of a maternal translocation resulting in partial monosomy 9p and partial trisomy 10p. The predominant phenotypic expression seen in this patient corresponds mostly to monosomy 9p syndrome. The second case is a 2-year-old boy with moderate developmental delay associated with a constellation of malformations evocating cranio-cerebello-cardiac syndrome (3C syndrome): corpus callosum and cerebellar hypoplasia, enlarged cisterna magna, ASD and peripheral pulmonary stenosis, hypertelorism and other dysmorphic features. FISH for subtelomeric regions revealed the unbalanced product of a paternal translocation resulting in partial monosomy 6p and partial trisomy 10q. We can speculate that a certain number of patients with characteristics of 3C syndrome may have a cryptic rearrangement involving one of these regions. Diagnosis of these cryptic rearrangements allowed genetic counseling and had a great impact on these families.

A girl with velocardiofacial phenotype and complex chromosome rearrangements involving 8p and 10p. L.K.

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We report a 4-year old girl with a velocardiofacial syndrome (VCF)-like phenotype and a de novo, apparently balanced complex chromosome rearrangement. She initially presented for assessment of velopharyngeal insufficiency due to hypernasal speech. She has distinctive facial features (long face, broad nasal bridge, and protuberent ears with simplified helices), bifid uvula, strabismus and joint laxity. She is developmentally delayed, with performance on language testing approximately 2 S.D. below the mean expected for age. She has significant difficulty with sensory regulation and perseverative behaviours. The karyotype is characterized by the presence of two derivative chromosomes; 46,XX,der(8)(10pter->10p12.32::8p12->8qter), der(10)(8pter->8p21.3::10p12.32->10p11.23::8p21.3->8p12::10p11.23->10qter). The der(8) is a result of translocation of the segment 10p12.32-pter onto 8p12. The der(10) has two 8p segments collectively from 8p12-pter in that the segment 8p21.3-pter is translocated onto 10p12.32 and the segment 8p12-p21.3 is inserted at 10p11.23. FISH analysis showed no microdeletion of the major locus at 22q11.2 nor for the minor locus at 10p13p14. This case suggests that in addition to identified loci at 22q11.2 and 10p13p14, aberrations at 8p12, 10p11.23 or 10p12.32 could lead to VCF phenotype.

Phenotypic and Molecular Cytogenetic Characterization of a Child With a Terminal Deletion of 7q [del(7)(q36.2)]. *J.G. Pappas¹, E. Reich¹, K. Allan¹, L.E. McMorrow², M. Perle², H.E. Wyandt³, J.M. Milunski³.* 1) Human Genetics Program, Dept of Pediatrics New York Univ, Sch of Med, New York, NY; 2) Cytogenetics Laboratory, Dept of Pathology, New York Univ, Sch of Med, New York, NY; 3) Center for Human Genetics and Dept of Pediatrics, Boston University School of Medicine, Boston, MA.

We describe a girl that presented in infancy with large midline cleft lip and palate, gastroesophageal reflux and chronic constipation. Clinical examination at one year of age revealed microcephaly, bifrontal prominence, midline cleft palate, short and narrow palpebral fissures, depressed and broad nasal bridge, hypertelorism, bifid tip of the nose, widely spaced inverted nipples and tapering fingers. The neurological examination and the developmental evaluation were normal. The gastroesophageal reflux and constipation were successfully treated with antacids. The family history was not contributory. Chromosome analysis of peripheral blood cells by G-banding was normal. Fluorescent in situ hybridization (FISH) was performed using DNA probes specific for the subtelomeric ends of each chromosome arm (Vysis, Downers Grove, IL). Results revealed apparent negative hybridization of the probe, pcp7, to the long arm of one chromosome 7 homologue in the proband. Hybridization with a painting library, specific for chromosome 7 (Vysis), painted both chromosomes 7 over their entire length. No evidence of hybridization of 7 sequences was seen elsewhere in the genome. Hence, these results indicate a deletion in the terminal band of 7q. High resolution G-banding revealed apparent terminal deletion in band 7q36.2. The parental karyotypes were normal. To our knowledge this is the first report of del(7)(q36.2). There are 19 published cases with larger terminal deletions of 7q (R.S. Verma et al. Clin Genet 41:82-86, 1992). The phenotypic features of our case were common features among the published cases. These features along with developmental delay constitute the deletion 7q syndrome. Our case has the smallest deletion associated with the features of deletion 7q syndrome. The critical region of deletion 7q syndrome is probably in the distal portion of 7q36.

Prenatal Diagnosis of an Inherited Heterochromatic Variant Chromosome 4. *A.L. Zaslav¹, J. Jacob¹, G. Pierno², A. Fougner², F.Y. Alexander², D. Blumenthal³, J.E. Fox³.* 1) Dept Pathology, Long Island Jewish Medical Ctr, New Hyde Park, NY; 2) SonoScan Genetic Services, Forest Hills, NY; 3) Dept Pediatrics, Schneider Children's Hospital, New Hyde Park, NY.

Heterochromatic chromosome polymorphisms have been extensively reported. The majorities are associated with C-band-positive regions located on chromosomes 1, 9, and 16. We report a prenatal case of an unusual heterochromatic variant on chromosome 4. Amniocentesis was performed on a 35-year-old white female for AMA. The karyotype was 46,XY,add(4)(q35)?. One chromosome 4 homolog had an additional dark band at the terminus of the long arm. Parental chromosome analysis revealed that the chromosome 4 was maternally inherited. The mother and fetus were both Q and C-banding positive and NOR and DAPI Distamycin staining negative. FISH using the TelVysion 4q telomere probe was positive, FISH using Y and 9 WCP (Vysis Inc., Downers Grove, IL), 13/21, 14/22, and 15 alpha-satellite (Oncor, Gaithersburg, MD) were all negative. High-resolution ultrasound at 19 weeks revealed no apparent fetal anomalies. Based on these findings, it appears that this chromosome 4 was a rare heterochromatic variant. Heterochromatic variants have been demonstrated to have no phenotypic effect on carriers. Additional FISH studies are in progress. Follow up and confirmation studies after birth will also be performed to characterize this extra genetic material. This case illustrates the importance of reporting unusual variant chromosomes for genetic counseling issues. To the best of our knowledge, this is the first report of a heterochromatic variant involving part of the long arm of chromosome 4 in a phenotypically normal mother and an apparently normal fetus.

Disease associated balanced chromosome rearrangements (DBCR): Report of 2 new cases. *V.S. Tonk¹, H.E. Wyandt², X. Huang², N. Patel¹, M. Kukolich¹, L.H. Lockhart³, G.R.V.N. Velagaleti³*. 1) Department of Pediatrics, Texas Tech University, Lubbock, TX; 2) Center for Human Genetics, Boston University School of Medicine, Boston, MA; 3) Department of Pediatrics, University of Texas Medical Branch, Galveston, TX.

Disease associated balanced chromosome rearrangements (DBCR) causing truncation, deletion, inactivation or overexpression of specific genes are instrumental in identifying and cloning several disease genes and are estimated to be much more common than anticipated. In one survey the minimal frequency of combined balanced de novo reciprocal translocations and inversions causing abnormal phenotype is estimated to be .17%, a six-fold increase compared to the general population suggesting a causative linkage between the abnormality and the observed phenotypic traits. Here, we report two new cases of apparently balanced de novo translocations resulting in developmental delay and dysmorphic features. Case 1 involves an 8.5-year-old female referred for mild developmental delay and autistic behavior. She was small with dysmorphic facial features, bifid uvula, pigmentary anomalies and hyperextensibility. Chromosome analysis showed a complex karyotype involving the chromosomes 3, 8 and 18. FISH studies with paint and telomere probes confirmed the complex nature of the karyotype 46,XX,t(3;18;8)(q13.1;q22.2;q21.2). Case 2 involves a 17-year-old male with severe developmental delay and mental retardation. He was small at birth and later diagnosed with profound hearing loss, partial blindness, and a seizure disorder. On examination at age 17 years, he was small with synophrys and gynecomastia. Chromosome analysis showed a reciprocal translocation 46,XY,t(3;7)(q26.2;p15.1). FISH studies with telomere and paint probes confirmed the diagnosis. Majority of the DBCRs are associated with mental retardation and since more than 70% of the genes are known to express in human brain, DBCRs offer a large-scale resource for karyotype-phenotype correlations.

Genetic proof of unequal meiotic crossovers in reciprocal deletion and duplication of 17p11.2. *C.J. Shaw, J.R. Lupski.* Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX.

A number of common microdeletion syndromes have been shown to result from non-allelic homologous recombination (NAHR) within region-specific low-copy repeats (LCRs). The reciprocal duplications are predicted to occur at the same frequency; however, probably because of ascertainment bias and expected milder phenotypes, only few cases have been described. We previously identified seven patients with dup(17)(p11.2p11.2), the reciprocal of the Smith-Magenis syndrome (SMS) deletion (Potocki et al, Nature Genetics 2000). In 90% of SMS cases, identical ~ 3.7 Mb deletions in 17p11.2 have been identified. These deletions are flanked by large ~200 kb highly homologous directly oriented LCRs - proximal and distal SMS-REPs. The third middle SMS-REP is inverted with respect to them and maps inside the commonly deleted genomic region. To investigate the parental origin and to determine whether the common deletions and duplications arise by NAHR between the proximal and distal SMS-REPs, we analyzed the haplotypes of 13 SMS families and four dup(17)(p11.2p11.2) families using microsatellite markers directly flanking the SMS common deletion breakpoints. Eight deletions are paternal and five are maternal in origin. Five of the deletions are due to an interchromosomal exchange event, while eight are due to an intrachromosomal event. Of the four dup(17)(p11.2p11.2) cases, two duplications are paternal and two are maternal. Two duplications are due to an interchromosomal exchange event and two are intrachromosomal. When pooled with previously published data on six dup(17)(p11.2p11.2) cases, seven of ten duplications are paternal and three are maternal in origin. Six of the duplications are due to an interchromosomal exchange event, while four are due to an intrachromosomal event. Our data indicate that reciprocal deletion and duplication 17p11.2 result from unequal meiotic crossovers. These rearrangements occur via both interchromosomal and intrachromosomal exchange events between the proximal and distal SMS-REPs and there appears to be no parental origin bias associated with both common SMS deletions and the reciprocal duplications.

Novel Subtelomeric Rearrangements of Human Chromosomes Detected by Telomere FISH. *R. Saleki, C. Bacino, J. Wu, M. Santini, R. Naeem.* Mol. & Hum. Genetics, Baylor Col Medicine, Houston, TX.

Fluorescent in situ hybridization technique using a complete set of telomere specific probes (Telomere FISH) is routinely used to detect cryptic submicroscopic rearrangements that are otherwise undetectable by conventional cytogenetic methods. By revealing novel subtelomeric rearrangements, Telomere FISH can contribute to our understanding of their clinical significance. Here we report four new cases with rare abnormal telomeric regions. We present two cases in which the derivative chromosomes contained two distinct telomeric signals in tandem. In one case the 16q telomere signal was observed adjacent to the 21q telomere signal on a derivative chromosome 21. In the second case the long arm of a derivative chromosome 10 contained signals for 18p telomeric probe distal to its intact 10q telomeric region. In both cases, the breakpoints appear to be distal to the telomeric probe region. Abnormal telomeres represent a de novo event or are inherited from a parent with balanced translocation. In rare cases, the same abnormal telomeres are present in an affected proband and an unaffected parent. These telomeric rearrangements are commonly interpreted as normal rare variants with no influence on the phenotype. We report two cases that represent rare trisomic variants of subtelomeric regions. In the first case, a child with mental retardation and her unaffected mother were trisomic for the 4p telomeric region. The second case was observed in amniotic cells where G-banding had identified a paracentric inversion of the long arm of one chromosome 4. Telomere FISH indicated that fetus and the healthy father were both trisomic for 1p and monosomic for 4q telomeric regions. We present a summary of the reported rare variant telomeres from our lab and others and propose that a more detailed investigation might be necessary before their influence on the phenotype of proband can be dismissed. Theoretically, genetic phenomena such as genomic imprinting, sex specific effect, and further loss of telomeric integrity are few possibilities that might explain the inconsistency of the phenotypic expression of an abnormal telomeric region in the so called rare variants.

Submicroscopic telomere deletions of chromosome 9q. *D. Stewart¹, E. Zackai¹, A. Huang¹, L. Medne¹, K. Russell¹, E. Rossi², R. Tenconi³, B. Anderlid⁴, M. Nordenskjöld⁴, K. Gripp⁵, L. Nicholson⁵, N. Spinner¹, I. Krantz¹ and cytogenetics.* 1) Division of Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) Biologia Generale e Genetica Medica, Univ di Pavia, Pavia, Italy; 3) Dipartimento di Pediatria, Univ. di Padova, Padova, Italy; 4) Dept of Molecular Medicine, Clinical Genetics Unit, Karolinska Inst., Stockholm, Sweden; 5) DuPont Hospital for Children, Wilmington, DE.

We report 3 new cases of the 9q telomere deletion, a novel chromosomal syndrome that has not been characterized. Patient 1 was a 2.5 y/o boy with developmental delay, relative microcephaly (small head relative to other growth parameters), hypotonia, coarctation of aorta with subaortic valve stenosis and a VSD. Dysmorphic features included coarse, flat facies, down-slanting palpebral fissures, a high arched palate, tented upper lip, and protruding tongue. Patient 2 was a girl with a DORV, mitral and aortic stenoses and a VSD. Dysmorphic features included relative microcephaly, bifrontal narrowing, arched eyebrows, a short, hirsute forehead, overfolded helices, small mouth and chin, a tented upper lip, high arched palate, flat occiput and a short neck with extra skin. She died at 8 weeks of complications from cardiac surgery. Autopsy revealed severe periventricular leukomalacia. No other structural abnormalities were found. Patients 1 and 2 had a de novo 46,XX, del(9)(qter) karyotype identified by telomere analysis. Patient 3 was a 2.5 y/o with acquired microcephaly, developmental delay, posterior plagiocephaly, epicanthi, high and wide nasal bridge and tip, right cryptotia, thickened left superior helix, tented upper lip, and micrognathia. Telomere analysis showed a de novo 46,XY,der(9)t(9;8)(qter;qter) karyotype. Review of 7 cases of 9q telomere deletions with our cases revealed that all patients had developmental delay, 3/10 had hypotonia, and 3/10 had relative microcephaly. Common dysmorphic features included hypertelorism, eyebrow and ear abnormalities, high arched palate, and tented upper lip. Cardiac malformations, seen in two patients, were severe. The deletion sizes and breakpoints of several patients are currently being determined in our laboratory.

Characterization of breakpoints on chromosomes 4 and 15 in a patient with Prader-Willi syndrome with an unbalanced translocation. *M.C. Varela, G.M.P. Lopes, C. Fridman, C.P. Koiffmann.* Dept Biol, Univ Sao Paulo, Sao Paulo, Brazil. mcvarela@ib.usp.br.

The Prader-Willi syndrome (neonatal hypotonia, poor sucking, hyperphagia, obesity and characteristic behavior) is caused by deletions of 15q11-q13, maternal uniparental disomy of chromosome 15 or imprinting defects. Chromosome 15 structural rearrangements have been described in ~5% of the patients with a typical or atypical PWS phenotype. A PWS patient with severe mental retardation and an unbalanced translocation $t(4q; 15q)$ was studied to characterize the extent of the deletions and to correlate with the severity of the phenotype. The patient was an 8 year-old boy, first child born at term by cesarian section when the mother was 32 and the father 35 years of age; birth weight was 2500g, birth length 50cm and neonatal hypotonia with poor suck were noticed. He walked at 46/12 years and hyperphagia and obesity began at 16/12 years of age. At 8 years of age he had severe mental retardation, behavioral problems and no speech; weight was 45kg (above 97 centile), height 1.22m (25 centile) and PC 53.5 (98-p-50 centile). Foot length was 13.5cm (below 3rd centile), whole hand measurement was 14cm (25 centile). Patient's karyotype was 45,XY,-15,der(4)t(4;15)(q35;q13) and FISH with probes D15S11/CEP15-D1521 (Vysis) detected the deletion. Both parents had normal karyotypes. The patient was diagnosed by SNURF-SNRPN exon 1 methylation assay, and the deletion extents were investigated by microsatellite analysis of markers D4S2924, D4S426, D4S1652 (4qter) and D15S1002, D15S1048, D15S1019, D15S165, D15S1031, D15S1043, D15S1010 (15q13-q14). The deletion on chr4q was distal to D4S1652 and on chr15 between D15S1031 and D15S1010. The severe phenotype in our patient could be attributed to the extent of the deletion larger than usually seen in PWS patients (between D15S1002 and D15S1048). Although the unbalance of the derivative chr4 cannot be excluded as another cause of the severe phenotype, the breakpoint was located in the subtelomeric region near the telomere, a region described as one with the lowest gene concentrations in the Human Genome. Support: FAPESP, CEPID, CNPq.

Apparently stable dicentric chromosome resulting from a whole chromosome translocation identified on an amniocentesis. *M.B. Smith¹, T. Gonzales¹, N. Casellas-Castillo², J.B. Ravnar¹, S. Schwartz³, S.A. Berend¹.* 1) Genzyme Genetics, Santa Fe, NM; 2) Santurce, Puerto Rico; 3) Case Western Reserve University, Cleveland, OH.

Chromosomes with two active centromeres are generally thought to be unstable throughout the cell cycle due to the breakage that occurs when the centromeres are attracted to opposite poles. Normal segregation can occur when the spindle apparatus from a single pole binds to both centromeres, pulling both centromeres to the same pole. Stable dicentric chromosomes generally either contain one active and one inactive centromere, or the centromeres are closely spaced and work together as a single centromere. Here we present a patient referred for amniocentesis due to a positive maternal serum screen indicating an increased risk for Down syndrome. Cytogenetic analysis revealed a dicentric chromosome resulting from a translocation between the short arms of one chromosome 14 and one chromosome 20, generating a chromosome that appeared to contain an entire chromosome 20 attached to the distal short arm of chromosome 14. The reciprocal product of this translocation was not present. After further investigation with fluorescence in situ hybridization, the derivative chromosome was found to contain centromeres from both chromosomes with ribosomal RNA present between them. The subtelomere of the short arm of chromosome 20 was not present on the derivative chromosome: 45,XY,dic(14;20)(p11.2;p13).ish dic(14;20)(D20Z1+,D20S1157-,rRNA+,D14Z1/D22Z1+). Parental chromosome analyses were normal. The patient elected to continue the pregnancy after a level four ultrasound showed no apparent anomalies; however, there were early signs of growth retardation. This translocation results in monosomy for the distal region of the short arm of chromosome 20. The phenotype for deletions encompassing this region is vague; however, clinical features reported for patients with larger deletions from band 20p12 include short stature, mental retardation with seizures, deafness, vertebral anomalies, tetralogy of Fallot, and optic atrophy. Such cases will help delineate the characteristics and stability of dicentric chromosomes.

Origin of X Chromosome in Familial 47,XXX: Evidence for Maternal Susceptibility for Recurrent Meiosis II

Non-disjunction. *O. Reish*^{1,2}, *T. Berryman*², *T.R. Cunningham*³, *C. Sher*¹, *W.S. Oetting*^{3,4}. 1) Genetic Inst, Assaf Harofeh Medical Ctr, Zerifin, Israel; 2) Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel; 3) Department of Medicine, University of Minnesota, Minneapolis, MN; 4) Institute of Human Genetics, University of Minnesota, Minneapolis, MN.

We determined the meiotic origin and the stage of non-disjunction of the extra X chromosomes in two sisters with 47,XXX chromosomal complements. Segregation of the X chromosomes in all family members was analyzed using X-linked short tandem repeat polymorphic (STRP) markers. Densitometric analysis of two STRP markers confirmed that both sisters had three copies of the X chromosome and the extra X chromosomes were maternally derived. Both sisters did not share the same maternal homologue suggesting that the recurrent trisomy is non-homologous X chromosome specific. Haplotype analysis demonstrated a reduction to homozygosity for all markers examined, covering the entire length of the X chromosomes in both sisters. These findings suggested that the extra X chromosomes have derived from meiotic II (MII) non-disjunction following a nulitransitional meiotic I (MI). A lack of X chromosome recombination in the extra chromosomes of both sisters suggests a maternal genetic defect leading to an erratic recombination at MI. As a recurrence of MII non-disjunction of X chromosome is rare, it is possible that for a second event to occur in maternal gametes, an additional risk factor such as increased maternal age, as occurred in the second sister, is required. This information may contribute to further understanding of mechanisms leading to X chromosome non-disjunction. It may also assist the clinician in more accurate counseling of couples with a previous X chromosome trisomy in future pregnancies.

Engineering chromosomal rearrangements in mice to model del(17)(q21q23) and Down syndrome. *Y.E. Yu¹, M. Morishima², A. Pao¹, Y. Qi¹, S. Rivera¹, A. Baldini^{1, 2}, A. Bradley^{1, 3}.* 1) Dept of Molec & Human Genetics; 2) Dept of Pediatrics (Cardiology), Baylor College Med, Houston, TX; 3) Sanger Institute, Cambridge, UK.

del(17)(q21q23) is a rare human deletion disorder with the phenotypes of craniofacial abnormalities, esophageal atresia, and heart defects, such as septal defects and overriding aorta (Marsh et al., *J. Med. Genet.*, 2000, 37, 701). Our group has generated many hemizygous deletions in the syntenic regions on mouse chromosome 11 using chromosome-engineering technology (Yu & Bradley, *Nature Review Genetics*, 2001, 2, 780). Similar heart defects were detected in mice that carry one of the deletions, which spans 5 cM and is located between Mpo and Chad. Importantly, mice carrying both the deletion and the reciprocal duplication were normal. Thus, these mutant mice can provide a model system for the genetic dissection of this human chromosomal region. Down syndrome is caused by human trisomy 21. Because of the existence of highly conserved linkage groups between human chromosome 21 and three segments of the mouse genome on chromosomes 10, 16, and 17, we have started engineering a new mouse model of Down syndrome by generating duplications of these segments. The selected endpoints of the duplications are located immediately outside the mouse chromosomal segments that are conserved on human chromosome 21. At this moment, we have nearly completed the duplication on mouse chromosome 10 and have also started constructing the rearrangement on mouse chromosome 17 in mouse embryonic stem (ES) cells. The endpoints of the duplication on mouse chromosome 16 have also been determined. The engineered ES cells will be used to establish mice with triple hemizygous duplications. This model can provide tissues for analyzing the developmental consequences of human trisomy 21 and can also be used for the development of novel therapeutical interventions. We also intend to engineer smaller overlapping duplications within the mouse chromosomal regions that conserved with the trisomic human chromosome 21 regions to identify the causative gene(s).

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Disease Associated Balanced Chromosomal Rearrangements in 2000 Cytogenetic Analyses. *K. Prabhakara, A. Radha Ramadevi.* Diagnostics Division, Centre for DNA Fingerprinting & Diagnostics, ECIL Road, Hyderabad 500 076, India.

Disease associated balanced chromosomal rearrangements (DBCRs), which disrupt, delete or inactivate specific genes have been instrumental in positional cloning of many disease genes. We report here DBCRs found in our 2000 cytogenetic analyses in the referred population. The cytogenetic analysis was done in GTG-banded metaphases and fluorescence in-situ hybridization (FISH) was carried out whenever needed for the molecular cytogenetic characterization of the abnormality. DBCRs was detected in four cases: de novo pericentric inversion of chromosome 4 in a boy with piebaldism and mental retardation; familial 9;11 translocation and XX sex reversal in a phenotypic boy with dysmorphic features, delayed development and cryptorchidism; 5;13 translocation in an oligozoospermic male; familial 11;21 translocation in a female child with regression of milestones and mental retardation. The frequency of DBCRs in this referred population was 0.2 percent. Identification of DBCRs has greater implication in the characterization of disease genes in breakpoint regions involved in the chromosomal rearrangement.

Correlation between cytogenetic, fluorescence in situ hybridization and clinical findings in two cases of ring chromosome 4. *C.E. Uria Gomez, R. Garcia Cavazos, D.G. Mayen Molina, M.J. Zavaleta Abreu.* Depto. de Genetica, Inst. Nal. de Perinatologia, Mexico D.F. Mexico.

The ring chromosomes are cause of malformations, mental and growth retardation. The presence of ring chromosome 4 is a rare event. When the ring is complete, subtelomeric sequences are intact, and this cytogenetic finding is described as ring syndrome. We present two cases of ring chromosome 4 and discussed the cytogenetic, FISH, and clinical findings. The first case is a 4 year-old boy who was born at term from healthy non consanguineous parents. At birth the patient present bilateral preaxial polydactyly. He showed growth retardation, prominent sagital suture, microcephaly, midfacial hypoplasia, hirsutism, narrow forehead, low-set ears and narrow palate. The second case is a 18 months boy who was born at term from healthy non consanguineous parents. He showed intrauterine growth retardation, microcephaly, bilateral microtia, septal defect ventricular, clinodactyly of fifth finger and psychomotor retardation. **METHODS.-** Cytogenetic studies were performed in peripheral blood lymphocytes. FISH was performed using telomeric probes for chromosome 4. **RESULTS.-** Chromosomal analysis in the first case showed 46,XY,r(4)(p16.3q35.2) in 81/100 metaphases observed. Mitotic instability of the ring was observed in the rest of the cells. With GTG bands it seems that there was not material lost, however only one signal corresponding to 4qtel was observed by using FISH, so the 4ptel region was deleted. Chromosomal analysis in the second case showed 46,XY,r(4)(p16q35) in 92/100 metaphases observed. Mitotic instability of the ring was observed in the rest of the cells. With FISH the signals of 4ptel and 4qtel were absented in the ring chromosome. **DISCUSSION.-** We excluded the presence of ring syndrome because the 4ptel region was deleted in the first case and 4ptel and 4qtel as well in the second. The clinical findings support this. Probably the clinical findings are the results of both, the deleted material and the instability of the ring. We did not exclude some association with the Wolf-Hirshhorn syndrome, since in both cases the 4pter region is deleted.

Three cases of rare inversion of chromosome in couples with recurrent abortion. *H.W. Seol¹, H.J. Koh¹, S.K. Oh^{1,2}, D. Hwang³, Y.M. Choi^{1,2}, S.Y. Moon^{1,2}.* 1) Cytogenetics Lab., Institute of Reproductive Medicine and Population, Medical Research Center, Seoul National University, Seoul, Korea; 2) Department of Obstetrics and Gynecology, College of Medicine, Seoul National University; 3) Hamchoon Women's Clinic, Seoul, Korea.

Since the advent of new banding technique, diverse chromosome variants have been detected as well as subtle structural rearrangements and inversion. The risk of abnormal pregnancy outcome in a inversion carrier is a consequence of the formation of gametes with partial duplication and partial deletion, which in turn result from a crossover occurring within the inverted segment during meiosis. These chromosome variants have been found in increased frequency in association with reproductive failure. In a couple with experience of five fetal losses, a maternal pericentric inversion of chromosome 5 (46,XX,inv(5)(p15;q11)) was ascertained through conventional cytogenetic analysis and fluorescent in situ hybridization (FISH) with LSI Cri-du-Chat(5p15.2) probe (VYSIS, USA). A paternal pericentric inversion of chromosome 9 (46,XY,inv(9)(p24q12)) was analyzed with TelVysion 9p probe in a couple with three fetal losses. And in a couple with two fetal losses, a maternal paracentric inversion of chromosome 13(46,XX,inv(13)(q13q22)) was confirmed with LSI 13(13q14) probe. Therefore couple who had experienced repetitive fetal losses have to be examined parental chromosomal analysis and genetic counseling such as prenatal diagnosis should also be considered in subsequent pregnancy.

Craniosynostosis and congenital heart anomalies associated with a maternal deletion of 15q15-22.1. *P.G. Wheeler, N. Shur, J. Cowan.* Dept Pediatrics, Div Genetics, Tufts Univ Sch Medicine, Boston, MA.

Interstitial deletions of 15q outside of the Prader-Willi/Angelman region (15q11.2-13) are uncommon. To date there have been 2 reports of individuals with paternal deletions in 15q15-22.1 with one case having craniosynostosis (del 15q15-22.1) and the other congenital heart disease (del 15q15.2-21.2). We report on a female infant with a maternal 15q15-22.1 deletion. This infant was noted at birth to have turribrachycephaly secondary to congenital craniosynostosis. In addition, a variant of tetralogy of Fallot was found as was unusual overlapping of the 3rd, 4th and 5th toes. She had a tall, prominent forehead, depressed nasal bridge and downslanting palpebral fissures. She had initial hypotonia and follow-up at 8 months indicates developmental delay. Karyotype analysis found a de novo maternal deletion of 15q15-22.1. Our patient shares some features with the previously reported paternal deletions of this area, but her problems are more severe than in either case. Since she is the first reported maternal deletion of this area, the possibility of imprinting as an explanation for her increased severity has to be considered. The presence of craniosynostosis in 2 individuals with this deletion also implies that an area critical for sutural function is in 15q15-22.1. The child with congenital heart disease and the 15q15.2-21.2 deletion did not have craniosynostosis implying that the critical area for sutural formation may be either in 15q15-15.2 or 15q21.2-22.1. There has been one report of a mutation in fibrillin 1 (15q21.2) leading to Shprintzen-Goldberg syndrome (craniosynostosis plus Marfanoid habitus). But, other individuals with deletions encompassing 15q21.2 have not been reported to have craniosynostosis and thus 15q15-15.2 may be the most likely region for a gene involved in suture function.

Molecular characterization of mosaic tetraploidy in chorionic villi tissue from a missed abortion. *J. Van Brunt*¹, *R. Mao*³, *A. Fauchaux*⁴, *M.M. Li*^{1,2}. 1) Hayward Genetics Center; 2) Department of Pediatrics, Tulane University School of Medicine, New Orleans, LA; 3) DNA Diagnostic Laboratory, University of Utah, Salt Lake City, UT; 4) Professional Medical Co., LaPlace, LA.

The generation of tetraploid cells through the failure of mitotic division is a common artifact in long-term cultures of chorionic villi. This culture-induced mosaicism makes it difficult to distinguish constitutional tetraploidy from pseudotetraploidy. When both the diploid and tetraploid cell lines are female, the interpretation is further complicated by the possibility of maternal cell contamination (MCC). A POC specimen from a missed abortion at 9 5/7 weeks gestation was received for cytogenetic analysis. Chorionic villi were cultured for chromosome analysis. Two hundred metaphases were analyzed and the results showed that 70% of them were tetraploid (92,XXXX), 24% diploid (46,XX), and 6% octaploid. In order to rule out the culture artifact, we carefully screened all the primary cultures and compared the percentage of tetraploid cells in this case with that of CVS cultures from a normal pregnancy (22 %) and published data (4-58%). Since tetraploid cells were present in all the primary cultures of this case at a much higher percentage, it is unlikely that these cells were due to culture artifacts. To rule out MCC and to explore the mechanisms of tetraploidy formation, we studied nine polymorphic DNA markers in DNA samples from the chorionic villi cultures and parental blood samples. Five of the nine markers were informative. The chorionic villi sample displayed two alleles, one maternal and one paternal, for each informative marker. These results ruled out MCC since this would result in 3 alleles for informative markers. Trispermy, or a diploid egg fertilized by a diploid sperm, is not likely to be the cause of the tetraploidy as this would result in 3 or 4 alleles for informative markers. Therefore, this constitutional mosaic tetraploidy is most likely the result of postzygotic duplication. Because the fetal tissue was not available for the study, we could not determine if the pregnancy wastage was caused by placental insufficiency only or by tetraploidy in both the placenta and the fetus.

A child having mosaicism for an extra ring chromosome 16 and normal development. *U.T. Sundaram¹, K. Turner¹, P. Papenhausen², J. Bodurtha¹, C. Jackson-Cook¹.* 1) MCV Campus of VCU, Richmond, VA; 2) LabCorp RTP, N.C.

Through the use of FISH the complete characterization of extra structurally abnormal chromosomes (ESC) has expanded, thereby allowing for the recognition of new clinical entities resulting from partial trisomic imbalances. We report a case with mosaicism for an extra ring chromosome 16. The female proband presented at 6 months of age with a unilateral transverse palmar crease and epicanthal folds, and was referred for genetic evaluation to rule out Down syndrome. Her growth and development were age appropriate. A standard GTG-banding chromosome study showed that she had a mosaic complement with an ESC in 20% (7 of 34 cells) of the lymphocytes scored. Based on FISH & GTG-banding, the marker was further defined as a ring [r(16)(p13.3q22)]. Parental chromosomes appeared normal. Follow-up studies at 2.5 years of age showed no developmental delay. Her height and weight were in the 90th percentile and she had no dysmorphic findings. A FISH analysis at 2.5 y.o., using cultured and uncultured lymphocytes, as well as buccal mucosa cells, showed no significant difference in the proportion of cells having the r(16), which was now present in only 1.4%, 3.5%, and 2.7% of cells, respectively. To date only 5 other cases of an extra r(16) have been reported, including 4 patients with mosaicism (3 ascertained prenatally; 1 postnatally) and 1 with an extra r(16) in all cells (postnatally ascertained). The clinical findings in these patients have varied, ranging from a normal phenotype (a child with 90% mosaicism) to severe mental retardation with microcephaly and other anomalies (a case with 75% mosaicism). The observed disparity in clinical outcome for these cases showed no clear correlation between phenotypic severity and mosaicism level and/or breakpoint involved in the rings. Thus, until more information is obtained to better understand the causes of the variation in phenotypic outcome, providing accurate genetic counseling for individuals having an extra r(16) will remain a dilemma.

Deletion 3p25 in a mother and child. *S. Sklower-Brooks¹, J. Pappas², C. Duncan¹, M. Genovese¹, H. Gu¹, E.C. Jenkins¹.* 1) NYS Institute for Basic Research Staten Island, NY; 2) Dept. of Pediatrics NYU School of Medicine New York, NY.

A mother and her infant with del(3)(p25) were identified due to dysmorphic findings in the infant. Born at 36 weeks by C-section due to breech position, to a G2P1 30 y/o, her birth weight was 2800g. Pregnancy was complicated by morning sickness. Because of tachypnea she was admitted to the NICU and craniosynostosis was considered but ruled out. A karyotype was requested. She was discharged on the third day of life. At 2 weeks of age she had mild facial asymmetry, overriding sutures, deep periorbital creases, epicanthal folds, mildly downslanted palpebral fissures and micrognathia. At 1 mo. her growth parameters were normal. Based on the literature, genetic counseling was provided to the family that the child would likely have significant developmental disability. The mother was subsequently found to have the identical deletion on chromosome 3. No evidence of a translocation was present by G-banding, FISH with wcp3 & a 3p telomere probe. The karyotype of the baby girl is 46,XX,del(3)(p25.3).ish 3(wcp3x2),del(3)(p25(D4S4559-))(mat). About 30 cases of 3p25 deletion have been reported. Classic features of the syndrome include, characteristic facies, microcephaly, mental and growth retardation. Tazelaar et al, 1991 reported on an affected mother and son with 3p25 deletion. Another case has been reported of a prenatally detected, parentally transmitted del3p25.3 with no phenotypic effect (Knight et al, 1995) and we recently learned of a similar case identified during infertility work-up in which the normal father carries the deletion (S.Fallet, personal communication). Despite extensive cytogenetic evaluation no cryptic translocation was detected in the mother, although this is certainly a possibility. This family illustrates a genetic counseling dilemma. Careful assessment of the breakpoint appears critical in the 3p-syndrome. These three cases likely have breakpoints distal to the genes responsible for the syndrome. Further molecular testing with probes from the deleted region might provide an explanation for the lack of phenotype. (Supported in part by the NYS Office of Mental Retardation and Developmental Disabilities).

Partial trisomy 13 due to a der(13)t(13;13)(p11.2;q22) translocation. A. Santiago-Cornier¹, S. Carlo¹, M. Borges², V. Franceschini¹, S. Kleyman³, M.J. Macera³, A. Babu³. 1) Genetics Division, Ponce school of Medicine, Ponce, PR; 2) San Juan Bautista School of Medicine, San Juan, PR; 3) Division of Molecular Medicine and Genetics, Department of Medicine, Wyckoff Heights Medical Center, Brooklyn, NY.

A 7 month old baby girl was referred for genetic evaluation because of a cleft palate, polydactyly of fingers, broad and adducted thumbs, broad big toes, borderline low set ears, thin lips, bulbous nose tip and mild hypertonicity. She was born to a 21 year old G3P2A1 mother after an uneventful pregnancy and vaginal delivery. Peripheral blood chromosomal analysis was performed to rule out suspected mosaic trisomy 13. Cytogenetic analysis revealed additional material in the short arm of chromosome 13 with band homology to the short arm (p) of chromosome 9 as well as the distal long arm (q) of 13. Fluorescence in situ hybridization (FISH) using whole chromosome paints (wcp) for 9 and 13 revealed that the additional segment was derived from 13. Based on G-band patterns and FISH observations the karyotype was designated as 46,XX,der(13)t(13;13)(p11.2;q22).ish der(13)(wcp9-,wcp13+). Parental peripheral blood has been requested to perform cytogenetic analysis to determine if the der(13) is a product of an unequal segregation of a familial balanced translocation or the de novo appearance of an unbalanced derivative chromosome. There have been about twenty reported cases of partial trisomy 13 due to a duplication of 13q22 to qter. The clinical findings included among others, prominent forehead, epicanthic folds, low set dysplastic ears, frequent polydactyly, high palate and inguinal hernias. Major malformations, described for full trisomy 13 were not common in these cases, however, cleft lip and cleft palate were reported. The mild presentation of this child prompted the initial diagnosis of a possible mosaic trisomy 13. The clinical features in this case are consistent with partial trisomy of 13 and are presented in an effort to elucidate phenotype and genotype relations.

Clinical, Somatic Cytogenetic and Molecular (AZF Microdeletions) evaluation of 133 men with Idiopathic Azoospermia or Oligospermia. *J.M. Pina-Neto¹, R. Bisinella¹, R.C.V Carrara¹, E. Sartoratto².* 1) Dept Genetics, Fac Medicine Ribeirao Preto, Sao Paulo, Brazil; 2) Molecular Genetics Center, University of Campinas (UNICAMP), Campinas, Sao Paulo, BRAZIL.

The objective was to investigate the frequency of somatic chromosome abnormalities and AZF microdeletions in patients with idiopathic azoospermia or oligospermia. Were studied 133 men evaluated at University Hospital of Faculty of Medicine of Ribeirao Preto, BRAZIL. The somatic karyotype was obtained by lymphocyte culture and analyzing 100 GTG metaphases and the AZF microdeletions were evaluated through PCR multiplex using 28 STSs specific for intervals 5 and 6 of the Yq11 region (Henegariu et al., 1994). In 13/133 patients (approx. 10%) we detected a somatic chromosomal anomaly (6 patients with 47,XXY karyotype, 2 with a mosaic 47,XXY/46,XY, 2 with a balanced 13q/14q translocations, one male 46,XX, one 45,X-11%;/46,X,+frag-89%, one 46,XY/47,XY,+i(22p)-10%. The microdeletions of AZF genes were detected in 9/133 patients (6.77%), with all AZF genes (a,b,c) involved in microdeletions. This work reinforces the importance of genetics studies before male submission to assisted fertilization techniques.

Three examples of complex chromosomal rearrangements (CCRs): Family studies and FISH analysis. *G.D. Wenger^{1,3}, E. Hamelberg¹, M. Millard¹, G.E. Herman², J.M. Gastier^{1,3}.* 1) Lab Medicine, Children's Hosp, Columbus, OH; 2) Pediatrics, Ohio State University; 3) Pathology, Ohio State University.

CCRs, defined as those involving 2 or more chromosomes and at least 3 breakpoints, are rarely seen in constitutional karyotypes. The probability that an essential gene is disrupted or that subtle gain or loss of sequences occurs during generation of the CCR increases with the number of breakpoints. Familial CCRs tend to have fewer breakpoints, involve fewer chromosomes (cs) than those that are not familial, and are transmitted frequently through females. We report two familial and one de novo cases of CCRs. CCR1 was identified in a newborn with multiple congenital anomalies. The mother (G13P7) had a 3 break rearrangement involving one cs 6 and both the short and long arms of one cs 10. Previous pregnancy loss specimens had two different unbalanced karyotypes. The infant's unbalanced karyotype included a rec(10). CCR 2, ascertained through a 1 year old child with macrocephaly, developmental delay, and deafness, involved an insertion and translocations between cs 2, 3, and 8. The mother and an older normal sib each had the balanced CCR in which 2p21-p25.1 was inserted into cs 8 at q24.1, and the segment distal to the insertion point was exchanged in an apparently balanced reciprocal translocation with 3p. The infant had an unbalanced cs complement including the der(3) and the der(8), and two normal cs 2 homologs, resulting in partial trisomy for 2p. CCR3 was identified in a newborn with cleft palate, brain abnormalities, and cardiac defect. Conception had followed insemination with donor sperm; a previous pregnancy conceived using the same sperm donor resulted in a normal offspring. The CCR involved cs 2, 5, 6, 8, and 10, with apparently balanced reciprocal translocations between 2q and 5q, and between the short arm of the same cs 5 homolog and 8q; an interstitial deletion of 6q; and an inverted duplication of 10p, resulting in partial trisomy for 10p and partial monosomy for 6q. The maternal karyotype was normal. These rare cases provide opportunities to observe meiotic behavior of complex rearrangements, as well as presenting challenges for analysis and nomenclature.

Construction of a natural panel of 11p11.2 deletions. *K. Wakui*¹, *L. Potocki*^{1,2}, *C.D. McCaskill*¹, *P.-L. Kuo*³, *M. Irons*⁴, *J.T. Hecht*⁵, *L.G. Shaffer*¹. 1) Molecular & Human Genetics, Baylor College Medicine, Houston, TX; 2) Genetics, Texas Children's Hospital, Houston, TX; 3) OB/GYN, National Cheng-Kung University Medical College, Tainan, Taiwan; 4) Genetics and Metabolism, Children's Hospital, Harvard Medical School, Boston, MA; 5) Pediatrics, University of Texas Medical School at Houston, Houston, TX.

The Potocki-Shaffer syndrome (PSS, OMIM#601224) is a contiguous gene deletion syndrome caused by deletion of multiple genes within the short arm of chromosome 11 [del(11)(p11.2p12)]. The full spectrum of PSS has been delineated to include mental retardation, multiple exostoses, biparietal foramina, minor craniofacial anomalies and genital anomalies in males. The presence of multiple exostoses is associated with deletion of the *EXT2* gene and the presence of biparietal foramina has been shown to be associated with deletion of *ALX4*, both within 11p11.2. Specific genes related to mental retardation or other abnormalities have yet to be identified. We constructed a BAC/STS map of 11p11.2-p12 including the *EXT2* and *ALX4* regions using eight deletion patient cell lines by FISH and STS analyses. The extent of the largest deletion is about 10 Mb. The smallest deletion includes only the *EXT2* gene. This patient has isolated multiple exostoses and not PSS. The most distal breakpoint in the PSS patients we analyzed is about 41.5 Mb from the p terminus and the proximal breakpoint is about 49.5-50 Mb from the p terminus (close to the 11cen). Seven patients have deletion of both *EXT2* and *ALX4* regions. One patient is deleted for *EXT2*, but not *ALX4* by FISH analysis. However, this patient has biparietal foramina, and disruption of *ALX4* at the proximal deletion breakpoint is suspected. Five patients from three families have no mental retardation. Thus, the gene(s) related to mental retardation may be located either between 45.7-50.0 Mb and/or 41.3-44.3 Mb from the p terminus. Further delineation of the breakpoints will refine the regions in which to search for the gene(s) causing the mental retardation and the craniofacial features of the syndrome and may uncover sequences susceptible to chromosome rearrangement.

Two Cases of Interstitial Duplications Detected Only by Interphase FISH. *P.R. Papenhausen¹, P. Singh-Kahlon¹, S. Griffin¹, P. Stone¹, K.K. Rogers², J.E. Rosini³, I.K. Gadi¹, J.H. Tepperberg¹, P.M. Mowrey¹.* 1) Dept Cytogenetics, Labcorp of America, Res Triangle Pk, NC; 2) Cytogenetics, UNC-Chapel Hill, Chapel Hill, NC; 3) REFUAH Health Center, Spring Valley, NY.

A seven-year-old boy was referred for microdeletion FISH (DiGeorge/VCF) secondary to coarctation of the aorta, microcephaly, hypotelorism, low set ears, and developmental delay (mild MR, 25th percentile height and weight). There was a language disorder with skills >3 yrs below normal for age. During the course of routine metaphase analysis using the D22S75 probe (Vysis, Inc.) it was apparent that three separate signals were discernable in 47/50 interphase cells, although normal signals were observed in metaphase analysis. High resolution G-banding follow-up showed normal appearing 22 homologues. A second familial study involved a 5-year-old boy referred for hyperactivity and aggressiveness and his 14-year-old MR sister. Both had a small increase in the size of the terminal 1p36.1 euchromatin. FISH for the subtelomere (CEB108/T7) was normal, but follow-up analysis for the more proximal (1p36.1) p58 and midi satellite probes showed that both of these loci were duplicated. The duplications were again apparent only in interphase analysis, demonstrating the enhanced resolution available at interphase. Interestingly, further familial studies of a normal sister and the borderline retarded mother also showed the apparent duplication. The mother had abnormal teeth and an elfin-like face, but this was not seen in the children. Continued studies of this family are planned to include maternal relatives. Cases with duplications of the commonly deleted segments in microdeletion syndromes have been expected from the postulated meiotic misalignment mechanism, but possibly because of mild phenotypes few have been detected. Another reason could be that these are also below the resolution of metaphase FISH. The associated heart defect in case 1 may initiate FISH analysis in similar cases and interphase vigilance may help reveal a new syndrome. Case 2 also demonstrates the utility of interphase FISH, but will require further familial follow-up to establish phenotypic effects.

Distal Xp deletion associated with unbalanced X-rearrangement in patients with dysmorphism, short stature and Leri-Weill dyschondrosteosis. *S.K. Tan¹, Y.S. Choy², A. Mekesat¹*. 1) Department of Cytogenetics, Kuala Lumpur Hospital, Kuala Lumpur, Malaysia; 2) Genetic Unit, Pediatrics Institute, Kuala Lumpur Hospital, Kuala Lumpur, Malaysia.

Very distal deletion of short arm of chromosome X results in the loss of SHOX gene and therefore short stature with or without Medelung deformity in an individual. However, the deletion may not be seen in routine karyotyping but would be strongly suspected in X-rearrangement involving the distal p arm of X chromosome particularly so if there is mesomelic dwarfism clinically. Here, we described 3 patients with such deletion proven by FISH using TelVysion XpYp spectrum green probe. The first patient is a 5 year old girl with severe short stature, Medelung deformity, dysmorphic facies, atrium septal defect, ocular albinism and global developmental delay associated with 46,X, der(X)t(X;17)(p22.3;p13). Another patient, a 2-year-old boy presented with similar problems had undescended testes in addition due to dup(X)(p22.1-22.3). The third patient was clinically a perfect girl with just short stature and complete female genital organs but karyotype showing der(X)t(X;Y)(p22.3;q12). IGF1 and GH levels after GH stimulation were normal.

How telocentric mouse chromosomes are. *C.C. Lin^{1,3}, Y.C. Li¹, C. Lee²*. 1) Department of Life Sciences, Chung Shan Medical University, Taichung, Taiwan; 2) Department of Pathology, Brigham and Woman's Hospital, Harvard Medical School, Boston, MA, USA; 3) Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada.

Telocentric is the term implying that a chromosome has a centromere located at the end of the chromosome arm. Unfortunately, this terminology has an inherent problem since no normal eukaryotic chromosome is truly telocentric. Mouse chromosomes in general were considered to be telocentric. However some recent publications still described them as telo/acrocentric. In this investigation, we used 4 DNA probes for multi-color FISH studies to determine how telocentric the mouse chromosomes are. These included mouse major and minor satellite DNAs, mammalian telomeric DNA and whole chromosome painting probes for mouse chromosomes 4, 5 and 13 specifically. Hybridization signals on metaphase chromosomes showed that mouse minor satellite DNA (centromeric DNA) was closely associated or co-localized with telomeric sequence in one of the terminal ends of mouse chromosome. Fiber-FISH study further demonstrated that mouse minor satellite DNA is organized as single contiguous array closely associated with telomeric DNA array in those specific mouse chromosomes examined. Our study suggested that a mouse centromere can be located within 10 kb from the telomere of the mouse chromosome. This study was supported by a grant from the National Health Research Institute (NHRI- EX91-8933SL) of Taiwan.

Isolation and identification of a novel centromeric satellite DNA family highly conserved in several mammalian species. *Y.C. Li¹, C. Lee², W.S. Chang¹, S.Y. Li¹, C.C. Lin^{1,3}*. 1) Department of Life Sciences, Chung Shan Medical University, Taichung, Taiwan; 2) Department of Pathology, Brigham and Woman's Hospital, Harvard Medical School, Boston, MA, USA; 3) Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada.

In an attempt to amplify cervid centromeric satellite II DNA from the genomes of Indian muntjac and Chinese muntjac, a pair of primers derived from the white tailed deer satellite II DNA clone (OvDII) yielded a prominent ~1 kb PCR product (in addition to the expected 0.7 kb satellite II DNA fragments) in both species. The ~1 kb products were cloned, sequenced, Southern blot analyzed and FISH-studied to reveal that the ~1 kb cloned sequences indeed represent a previously unknown mammalian satellite DNA family which now designated as cervid satellite IV DNA. ~1 kb PCR clones were also obtained from the genomes of the black tailed deer and Canadian woodland caribou with similar primer pairs. Extremely high sequence conservation (over 90% homology) was observed among the clones generated from all four deer species and PCR-Southern hybridization experiments further verified the co-amplification of two kinds of satellite DNA sequences with a same pair of primers. This satellite DNA was found to co-localize with CENPs at the kinetochore by simultaneously FISH and immunofluorescence study. Due to its highly sequence conservation and close association with kinetochores, the newly identified satellite DNA may have a functional centromeric role. This study was supported by a grant from the National Health Research Institute (NHRI-EX90-8933SL) of Taiwan.

Chromosome-specific variation in telomere lengths & somatic cell aneuploidy acquired with aging. *R. Rehder, L. Corey, C. Jackson-Cook.* VCU, Richmond, VA.

Two proposed hallmarks of genomic instability are telomere shortening and increased chromosomal aneuploidy. To test the hypothesis that somatic cells acquire genomic changes as one ages (>50 y.o.), we compared: (1) aneuploidy levels in cells from different tissues (lymphocytes & buccal mucosa cells) and growth conditions (cultured vs. uncultured lymphocytes) using interphase FISH; (2) chromosome arm-specific telomere lengths (using a semi-quantitative Q-FISH based method); and (3) chromosome-specific acquired aneuploidy levels to telomere lengths. To date, we have scored the above attributes in 15 females (ages 52-72). In all cell types and females studied, chromosomal loss exceeded gain, with chromosomes having large heterochromatic regions (qh+) being lost with the highest frequency [chromosomes 16 (4.2%) and 9 (3.4%) based on both CEP & distal long arm probes; thus showing true loss rather than fusion of the qh+ regions], followed by chromosomes 17 (3.1%); X (2.7%); and 1 (2.6%). Differences in aneuploidy frequencies (loss but not gain) were also seen between tissues, with loss of chromosomes 1 and 17 being significantly lower in buccal mucosa cells compared to lymphocytes ($p < 0.01$). Cultured and uncultured cells also differed, with X chromosome loss being nearly twice as frequent (4.6% vs. 2.7%) in the cultured cells ($p < 0.01$). For chromosome-specific telomere lengths, differences were seen between females and chromosomes ($p < 0.0001$), with 21p, 14p, and 22p having the longest, while 4p and 2q had the shortest telomeres. This latter finding is of particular interest given recent reports of a variant for a deletion of the 2q subtelomeric region. As expected, average telomere length decreased with increasing age. Although some individuals showed a clear trend toward increased acquired aneuploidy levels with shortened telomere length (p or q arm), no consistent pattern between these traits has yet emerged. In summary, we detected individual and chromosome-specific differences in acquired aneuploidy frequencies and telomere lengths. This information could be helpful for designing tests (mutagen, health or pharmacogenetic applications) to screen for individual differences in susceptibility to change in the human genome.

Spectral karyotyping on 25 constitutional marker chromosomes. *P. Guanciali Franchi*^{1,2}, *E. Morizio*^{1,2}, *D. Fantasia*^{1,2}, *M.M. Rinaldi*⁴, *L. Cristini*³, *A. Simonelli*³, *A. Giannotti*⁵, *F. Lonardo*⁶, *A. Turci*⁷, *A. Zatterale*⁸, *M.G. Ciavarella*^{1,2}, *R. Mingarelli*⁹, *L. Stuppia*^{1,2}, *G. Calabrese*^{1,2}, *G. Palka*^{1,2}. 1) Scienze Biomediche, University of Chieti, Chieti; 2) Serv Genetica Umana, Pescara Hospital, Pescara; 3) S. Citogenetica, Frosinone; 4) Genetica Medica, Ospedale Cardarelli, Napoli; 5) Genetica Med, Ospedale Bambin Gesù, Rome; 6) Genetica Medica, Benevento Hospital, Benevento; 7) Citogenetica, Ravenna Hospital, Ravenna; 8) S. Citogenetica ASL-NA1, Napoli; 9) CSS-Mendel, Rome, Italy.

Constitutional marker chromosomes (MCs) are often associated with mental retardation and phenotypic abnormalities. Their characterisation is often difficult, expensive and time consuming, especially when MCs are found in prenatal diagnosis. Spectral karyotyping (SKY) has allowed a precise and rapid classification of chromosomal rearrangements with a single hybridisation of 24 chromosome painting probes. We report on a study of 25 cases of MCs found at pre- or postnatal diagnosis using SKY analysis. Eleven cases were detected at amniocentesis and 14 in postnatal tests performed for repeated miscarriages, or congenital malformation with or without mental retardation. In 10 cases SKY analysis allowed to classify MCs as der(22), in 7 of which with normal phenotype, while in 1 case associated with repeated abortions, in 1 with mental retardation, and in the last patient with anal atresia and cat-eye syndrome critical region duplication. Five cases showed MCs which SKY defined as invdup(15). In 3 of these there was normal phenotype with no involvement of Prader-Willi/Angelman syndrome (PWS/AG) critical region, while in the other 2, duplication of PWS/AG critical region was found by FISH on invdup(15) associated with mental retardation and other dysmorphisms. The remaining cases, all phenotypically abnormal, showed involvement of chromosomes 2, 3, 5, 13, 16, and 18. SKY analysis provided a fast and reliable tool for the identification of all MCs, highly important in the eleven MCs cases found at amniocentesis. Moreover, in combination with FISH using locus specific probes, SKY approach allowed a finer clinical and prognostic evaluation of patients, reducing genetic counselling dilemmas.

Ring chromosome 4 with deafness, developmental delay, and type 2 diabetes mellitus. *P.R. Blackett, S. Li, J.J. Mulvihill.* Pediatrics, University of Oklahoma, Oklahoma City, OK.

Ring formation of chromosome 4 usually has a predominant loss of distal 4p including the Wolf-Hirschhorn syndrome region [WHS]. We describe a case with r[4] in a girl who presented without features of WHS; she had mild developmental delay, deafness, short stature, obesity, and onset of type 2 diabetes in adolescence. Karyotype analysis revealed a monocentric ring chromosome 4 in 20 cells, 46XX,r[4][p16q35]. Parental karyotypes were normal. FISH analysis with telomeric 4p and 4q probes failed to detect 4p and 4q signals. A DNA probe corresponding to the WHS region showed 2 copies of the signal. Hybridization with the FGFR3 probe revealed a single band with the same intensity in samples from the patient and patient's mother excluding a deletion. We conclude that the 4p junction was distal to the WHS and FGFR3 loci but proximal to the D4S3360 marker. The 4q breakpoint was close to the telomere. The phenotype seems different from previous patients with 4p- or r[4], which have had more distal 4p deletion. The phenotype including skeletal findings and early onset of type 2 diabetes is inconsistent with other cases with 4p deletions. The findings suggest that the selective absence of distal genes on 4p could have lead to the phenotype including obesity and/or type 2 diabetes.

A complex family history and unusual FISH telomere findings. *E. Mak-Tam, W.S. Meschino, D. Allingham-Hawkins, A. Angelo, E. Capua, A. Mok, G. Rasiuk, P.R. Wyatt.* Dept Genetics, North York General Hosp, Toronto, ON, Canada.

The proband was referred to the genetics clinic at 3 years and 9 months. She had dysmorphic features, microcephaly, global developmental and language delay. She had deep-set eyes, a prominent nose with wide nasal bridge and mild micrognathia. She was similar in appearance to her mother who also was dysmorphic, microcephalic and significantly delayed. A four-generation pedigree showed an extensive maternal family history including schizophrenia and developmental delay with symptoms in the maternal grandmother and almost all maternal aunts and uncles of the proband. Only one maternal aunt has a normal phenotype. Chromosome, Fragile X and FISH for 22q11.2 results were all normal. FISH using the VYSIS telomere Xp/Yp probe showed an additional signal on the q terminal of one X chromosome in addition to the normal Xq telomere. This cryptic subtelomeric duplication of Xp (or homologous Yp sequences) was confirmed with probes from a second manufacturer (Cytocell). This unusual X chromosome was also present in the proband's mother. A maternal first cousin presented with developmental delay, speech problems and ADHD was also seen in the clinic. His karyotype was found to be 47,XYY. Unexpectedly, both he and his clinically normal mother were found to carry the same duplicated X. It is not clear if this X is clinically significant or a familial variant. One ambiguous finding is the presence of the duplicated X in both the proband's affected mother and her normal sister. A possible explanation is differential X inactivation in the two sisters. X inactivation studies have been recommended. Analysis of additional family members may also be informative. Inversion of an X chromosome in a family member from a previous generation may be a possible origin of this duplicated X. Neither VYSIS nor Cytocell report the existence of a variant such as the one described and no similar cases could be found in the literature. Documentation of similar cases is important to further our understanding of clinically significant subtelomeric changes and benign polymorphic variants.

Trisomy 17p resulting from isochromosome formation and whole arm translocation. *C.D. Martinhago¹, F.R.P. Souza², V.M. Motta³, A.C.C. Nassr³, L.A.F. Laureano¹, E.S. Ramos^{1,2}, L. Martelli^{1,2}.* 1) Medical Genetics, HCFMRP, University of Sao Paulo, Ribeirao Preto, SP, Brazil; 2) Dept.Genetics, School of Medicine of Ribeirao Preto - University of Sao Paulo, Brazil; 3) Division of Genetics, Sinha Junqueira Maternity, Ribeirao Preto, SP, Brazil.

Short arm isochromosome formation concomitant with translocation of an entire long arm is a rare mechanism of establishing of partial trisomy. We present a case of trisomy 17p and translocation 15p;17q. The propositus, a female newborn, is the second child born to young healthy nonrelated couple. There was no family history of congenital malformations. She was delivered by caesarian section, weighting 2,370g, showing a single umbilical artery, respiratory distress and gastroesophageal reflux. The physical examination revealed brachycephaly, triangular facies, prominent eyes secondary to hypoplastic supraorbital ridges, short and upward palpebral fissures, prominent nasal bridge with short nose and long philtrum, posteriorly rotated ears, micrognathia, webbed neck, widely spaced nipples, long fingers, clinodactyly of 5th finger, bilateral transversal palmar crease and muscular hipotrophy. PDA was diagnosed by echocardiogram. On recent examination (11 months), she presented psychomotor retardation and marked growth deficiency. Cytogenetic studies performed on peripheral lymphocytes using GTG, CBG and high resolution bandings revealed trisomy 17p due to an isochromosome 17p and a concomitant translocation between chromosomes 15 and 17. The parental studies were normal. Double-colour FISH using 17 telomeric probes and 15q plus 17 Whole Chromosome Painting probes confirmed the origin of the isochromosome and the chromosomes involved in the rearrangement. The final karyotype was 46,XX,-15,-17,+der(15)t(15;17)(p13;q11.2),+i(17)(p10). Isochromosomes can contain two identical and one nonidentical copy of the trisomic segment, representing an example of partial uniparental isodisomy with phenotypic consequences. In this case, molecular and nerve conduction studies become obligatory to confirm the early diagnosis of CMT1A disease, mapped on 17p11.2. Supported by: FAEPA-HCFMRP, University of Sao Paulo.

Dysmorphic features, extra chromosome material and multi-color FISH. *M.M. Li^{1,2}, K.E. Jackson^{1,2}, K.C. Kim¹, H. Andersson^{1,2}.* 1) Hayward Genetics Ctr; 2) Department of Pediatrics, Tulane Univ School Med, New Orleans, LA.

The presence of extra chromosome material of unknown origin is one of the major causes of birth defects. Accurate characterization of these unbalanced chromosome rearrangements is critical in clinical diagnosis and genetic counseling. Here we present two patients with dysmorphic features: 1) a de novo supernumerary marker chromosome (SMC) 5 and 2) a de novo partial trisomy 2p and small accompanying 18q deletion characterized by multi-color FISH (M-FISH). Case 1 was born at 34 weeks gestation. She was noted at birth to have epicanthal folds, a flattened nasal bridge, small ears and short fifth digits. Cardiac ECHO uncovered a secundum ASD. At age 18 months, she has demonstrated motor and cognitive delay. Cytogenetic studies revealed a karyotype of 47,XX,+mar[15]/46,XX[5]. M-FISH showed that the marker was derived from chromosome 5. The marker was further defined by FISH using a whole chromosome 5 painting probe and chromosome 1/5/19 a-satellite probe to be composed of the chromosome 5 centromere and the pericentric region. Case 2 was referred for chromosome study because of "Edward syndrome-like" dysmorphic features and severe development delay. Cytogenetic studies revealed a karyotype of 46,XY,add(18)(q23). M-FISH showed that the extra material was from chromosome 2. FISH with chromosome 2 telomere probes and the *N-myc* probe confirmed that the extra material originated from 2p and that the patient had three copies of the *N-myc* gene. Marker chromosomes derived from chromosome 5 are rare; only two cases have been previously reported and both are de novo and mosaic. The common features include epicanthal folds, flat faces, abnormal ears and development delay. Over 50 cases of partial trisomy 2p with or without an accompanying deletion have been reported. The common anomalies include a high forehead, wide depressed nasal bridge, hypertelorism and musculoskeletal defects. At least four cases of partial trisomy 2p associated with neuroblastoma have been reported indicating that germline duplication of 2p, giving rise to three copies of the *N-myc* gene may have predisposed the patients to the development of neuroblastoma.

Prenatal diagnosis of de novo balanced translocation between chromosome Y and 11. *Y.M. Kim¹, J.M. Kim¹, M.H. Lee¹, H.M. Ryu², S.Y. Park¹.* 1) Lab Medical Genetics, Samsung Cheil Hosp, Seoul, Korea; 2) Obstetrics and Gynecology, Samsung Cheil Hosp, Seoul, Korea.

We report a de novo balanced translocation between chromosome Y and 11 in prenatal diagnosis. The patient was referred for amniocentesis due to increased risk for Down syndrome at 16 wks of gestation. Karyotype of fetus revealed 46,X,t(Y;11)(q12;q23.2) by high-resolution GTG and RBG banding. The parents have a healthy girl and their karyotypes were both normal. Amniocentesis and percutaneous umbilical blood sampling were done at 21.2 wks of gestation for FISH (fluorescence in situ hybridization), CGH (comparative genomic hybridization) and PCR. FISH confirmed the breakpoint at the Yq12 region and CGH did not show any chromosomal amplification or loss. The targeted fetal sonography finding showed no definite evidence of fetal congenital anomaly with normal male external genitalia. DNA analysis using Y specific STS revealed the presence of SRY loci and euchromatic portions of the long arm including AZFa, b and c. Extensive counseling on potential of infertility and other congenital abnormalities was done. The parents wanted to continue the pregnancy and further examination will be followed.

FISH of kidney touch preparation: a useful approach to chromosome studies of products of conception. *J. Xu, J. Bourgeois.* Pathology and Molecular Medicine, and Laboratory Medicine, Hamilton Health Sciences and McMaster University.

We report use of FISH of touch preparation in analysis of 2 unusual cases of intrauterine fetal demise (IUFD). FISH with probes (AneuVysion, Vysis) specific for chromosomes 13, 18, 21, X, and Y was performed on intact nuclei from touch preparations of kidney section of the abortuses. Case 1 was of a 32 week gestational age IUFD with phenotypic features of triploidy and advanced maceration. Attempt to culture the macerated skin and tendon tissues failed, giving rise to no results from the routine cytogenetic method. FISH analysis of 50 interphase nuclei for each probe showed at least 86% of the cells had 3 representative signals, confirming clinical diagnosis of triploidy. Case 2 was an IUFD at 20 weeks gestational age in a 41 year old women. Only an external fetal and placental examination was granted. The external phenotypic features were suggestive of trisomy 21. G-banding analysis of 91 cells from a long-term (30-day) amnion culture showed a mosaic for 2 female cell lines, 46,XX (22%) and 92,XXXX (78%). FISH was performed to rule out possibility that the tetraploid cells result from a long-term culture. It showed that at least 84% (42/50) interphase cells for each probe had signals indicative of diploidy and less than 6% of the cells had signals indicative of tetraploidy. This finding indicates that the tetraploid cells seen in G-banding and FISH likely a result of technical artifact and background noise, respectively. The normal female karyotype is likely representative of the fetus. Our results show that FISH of kidney touch preparation could be invaluable for chromosome studies, genetic counseling and clinical management of those cases with clinical indication of common numerical aberrations that 1) have advanced maceration and very low likelihood of successful culture; and/or 2) need additional information for more accurate interpretation of a routine result.

FISH Analysis of Female Carriers of X-linked Chronic Granulomatous Disease Deletions. *K.C. Simon¹, D. Noack², J. Rae³, J.T. Curnutte³, V. Kolev¹, J.K. Blancato¹.* 1) Oncology, Georgetown University, Washington, DC; 2) The Scripps Research Institute, La Jolla, CA; 3) DNAX Research Institute, Palo Alto, CA.

Chronic granulomatous disease (CGD) is a rare inherited disease in which microbial killing ability is impaired due to a deficiency in NADPH oxidase activity. Proper functioning of NADPH oxidase is essential for reduction of oxygen to superoxide (O₂⁻) and conversion to compounds that kill bacteria and fungi (1). CGD patients are diagnosed at an early age and usually present with recurrent, severe infections and in many cases development of granulomas (2). Diagnosis of probands is made by measuring oxidative burst using dihydrorhodamine (DHR) assays in which oxidative burst capabilities of circulating neutrophils and monocytes are detected through the conversion of DHR to fluorescent rhodamine 123 (3). CGD is inherited as an X-linked or an autosomal recessive disorder with an incidence of 1/200,000 live births (2). All forms involve mutations of one of the four subunits of NADPH oxidase (p67*phox*(1q25), p47*phox*(7q11.23), p22*phox*(16q24) and gp91*phox*(Xp21.1)). X-linked transmission accounts for 70% of all CGD cases and is caused by a variety of mutations in the gp91*phox* subunit(CYBB) of cytochrome b558 (1). Carriers of large deletions in CYBB are undetectable by DNA sequencing; therefore we developed a FISH assay for carrier analysis of females and molecular confirmation of affected probands with deletions of exons 2-13 of the CYBB gene. FISH cosmid contig probes from PCR products of CYBB were tested on control lymphocytes and samples from families with possible CYBB deletions based on DHR analysis and lack of PCR amplification with CYBB specific primers (spanning the coding region) in the probands. We analyzed 4 carriers and 3 normal female samples from families to assess deletion of CYBB and were able to reliably determine carriers from non-carriers using the FISH contig probe to this region. This assay will represent a novel approach to screening carriers whose mutations may otherwise go undetected. 1. Segal et al. 2000. *Medicine (Baltimore)* 79:170-200 2. Winkelstein et al. 2000. *Medicine (Baltimore)* 79:155-169 3. Woodman et al. 1985. *Blood* 85:231-241.

Chromosome Specific Aneuploidy Rates in Pregnancy Loss: Use in Development of FISH Protocols When Tissue Culture Fails. *E. Sicinska*^{1,2,3}, *A. Thomas*¹, *P.M. Miron*^{1,3}. 1) Dept Pathology, Brigham & Women's Hospital, Boston, MA; 2) Dept Cancer Biology, Dana-Farber Cancer Institute, Boston, MA; 3) Harvard Medical School, Boston, MA.

Cytogenetic analysis of pregnancy loss is often crucial in determining cause and assessing future risk. In cases for which assessment of metaphase chromosomes is not possible, FISH can be a powerful adjunct for detection of targeted numeric abnormalities. While interphase FISH has the advantage of forgoing specimen culture, this analysis requires selection of probes to target particular chromosomes of interest. Ideally, the selection of probes should be optimized to minimize the number of hybridization reactions used to detect an abnormality. Frequently, the only clinical data available for a specimen are gestational age and maternal age. It is well established that gestational age contributes to the distribution of chromosome abnormalities as this distribution is significantly different in first trimester losses than in late second trimester and third trimester losses. However, the probability of a particular chromosome abnormality for a given gestational age has not been well defined. We determined the distribution of numerical chromosomal abnormalities by gestational age among 877 specimens shown to have at least one numeric abnormality by analysis of G-banded metaphase chromosomes. Our data show that the distribution of anomalies does change significantly throughout the first 15 weeks of gestation. For example, trisomy 16, the most common abnormality in early losses, comprised more than 40% of the abnormal specimens at 6 weeks gestation, but only 10% of specimens at 12 weeks gestation. The percentage of trisomy 22, the second most common abnormality in early losses, remained fairly constant over the first 12 weeks gestation, comprising 10-15% of the abnormal specimens. Trisomy 15 was the third most common aneuploidy until 10 weeks gestation when it was then surpassed by trisomy 21. Our data suggest that multiplex FISH, when used for aneuploidy detection, should incorporate varying probe cocktails depending on the developmental age of the pregnancy loss.

Juxta-subtelomeric small deletions: a cytogenetic pitfall? *D. Sanlaville, M. Prieur, O. Raoul, M.C. De Blois, P. Gosset, T. Attie, A. Munnich, S.P. Romana, M. Vekemans, C. Turleau.* Dept Genetics, Hopital Necker Enfants Malades, Paris, France.

Subtelomeric imbalances are found in 6% of patients with unexplained mental retardation and a normal karyotype at a 550-bands level. However, in these patients, high-resolution karyotypes and comparative genomic hybridization (CGH) have also shown a significant number of interstitial imbalances. We recently observed using high resolution banding an unexpected number of apparently terminal deletions in patients with unexplained mental retardation. These small deletions concerned various chromosome ends: 2q, 4p, 5p, 6p, 6q, 8p, 9p and 20q. Clinical and cytogenetic data will be provided. In all these cases, FISH using commercially available subtelomeric probes was normal. Interstitial deletions were subsequently confirmed using either band specific probes or microsatellite markers. In conclusion, juxta-subtelomeric chromosome imbalances are a significant cause of mental retardation. They may be missed if suboptimal banding analysis is considered acceptable in conjunction with commercially available subtelomeric FISH screening. Suspected terminal deletions must not be rejected on the basis of a normal subtelomeric FISH analysis. Clinical evaluation and high resolution karyotypes are key-factors for the detection of small chromosomal imbalances. Moreover, it is important to consider juxta-subtelomeric imbalances when setting diagnostic tools such as CGH microarrays.

Delineation of cryptic subtelomeric duplications: Implications for an under-reported but important abnormality.

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Over the past few years, several studies have shown the importance of cryptic deletions involving the subtelomeric regions of chromosomes. These studies have revealed that approximately 2-8% of cases with unexplained MR/developmental delay, without a cytogenetic abnormality detected with routine analysis, could be due to a subtelomeric deletion. However, during this same time there have been very few reports examining the role of subtelomeric duplications. Over the past 10 years we have studied over 100 cases of de novo duplications, to better understand the phenotype-karyotype correlations and implications of different duplications. Utilizing both high-resolution chromosomal analysis and metaphase and interphase fluorescence in situ hybridization, with single copy BAC probes, we have identified 7 cryptic subtelomeric duplications (involving 6p, 11p, 8p, 16p, 18q and Xq). The majority of these range in size from 2-5 Mb, are usually within 3Mb of the telomeric region of the chromosome, and could not be detected without a combination of high-resolution chromosomes and FISH. To better understand these cases we have developed BAC maps characterizing each duplication and in two cases have identified duplication of specific genes accounting for the phenotypic abnormalities.

Based on the findings from these studies we have gained additional insights concerning the implication of duplicated material and can conclude the following: (1) similar to cryptic deletions, we have shown that there are also clinically relevant cryptic subtelomeric duplications; (2) because of the difficulty in detecting these duplications, they are likely to be under-ascertained; (3) BAC contigs constructed from the human genome data indicates that most of our duplications are less than 5 Mb; and (4) in two cases a further analysis of the duplicated region has elucidated genes responsible for the phenotypes.

A Case of 22q13.1 Deletion as a Result of Asymmetrically Dicentric 22. *Y.M. El-Gohary¹, U. Surti^{1,2}, E. McPherson^{1,2}, J. Hu^{1,2}, S. Kochmar¹.* 1) Genetics Department, UPMC Magee-Womens Hospital, Pittsburgh, PA; 2) University of Pittsburgh, School of Medicine, Pittsburgh, PA.

The patient was the 2750 gram, 50 cm, OFC 34 cm, male product of an uncomplicated full term pregnancy in a 30 year old G4 P3 mother. Chromosome studies were done because of minor anomalies including camptodactyly, mild micrognathia, minor asymmetry of the ears, inability to extend the fingers and overlapping toes. The karyotype of the patient is 46,XY,dic(22;22)(q13.1;q11.21). The derivative bisatellited dicentric chromosome 22 occurred denovo, as it was not found on analysis of parental bloods. Fluorescence In Situ Hybridization (FISH) using paint probes (Vysis Inc.) revealed positive signals covering the entire derivative dicentric #22 chromosome. Also FISH analysis was done using the DiGeorge chromosome probe 22q11.2 region (TUPLE1) and a marker probe 22q13.3 region (ARSA). The normal #22 chromosome showed the presence of both the critical region (TUPLE1) and the marker region (ARSA). The derivative dicentric #22 chromosome was found to contain one signal of the critical region (TUPLE1) but the absence of the (ARSA) marker region. Because the Stalk and Satellite areas on either side of the derivative #22 chromosome were not identical, this derivative chromosome more likely resulted from an unbalanced translocation between the q arms of two #22 chromosomes resulting in a partial monosomy for the segment 22q13.1 to qter, and a partial trisomy for a very small segment of 22pter to 22q11.21. On follow-up at one month, the patient's facial appearance remained mildly unusual, due mainly to his small chin, the hands remained somewhat clenched and his feet had improved since birth. He was growing normally, but delay is expected based on reports of other patients with deleted 22q13. In previous reports, the phenotype of distal del 22q has been non-specific with normal to accelerated growth, minor facial anomalies, hypotonia and developmental delay. Our patient did not show any features of trisomy 22 or Cat Eye syndrome, presumably because there is little euchromatin in the region of partial trisomy.

Mosaicism and endometrial adenocarcinoma in an adult Turner syndrome patient. *B.K. Goodman, V. Golembiewski-Ruiz, C.A. Tirado, P.S. Kishnani, T.T. Stenzel, J.M. Lancaster, S.J. Robboy.* Depts of Pathology, Pediatrics and Obstetrics and Gynecology, Duke Univ Medical Ctr, Durham, NC.

A 35-year-old female with Turner syndrome presented with a one-month history of significant GI symptoms, weight loss and an enlarged uterus. A CT scan showed ascites, omental thickening and a right adnexal mass. The patient was on hormone replacement, taken since 13 years of age. Following a genetics consult, peripheral blood was sent for chromosome analysis to exclude the presence of a Y-bearing cell line that could predispose to gonadoblastoma. The patient underwent gross tumor removal and surgical staging. Her karyotype was mosaic 45,X[13]/46,X, idic(Y)(q11.2)[37]. FISH analysis further defined the character of the small isochromosome, showing signals for SRY (Yp11.3) at each end, the presence of two copies of Y centromeric (alpha-satellite) sequences and the absence of satellite III sequences (Yq12). Thus, a Y-derived chromosome was present that contained only the most proximal long arm sequences, which could include the region implicated in predisposition to gonadoblastoma. FISH analysis also detected a cell line with two X chromosomes in 2% of nuclei, not detected by chromosome analysis. Pathologic examination showed a primary endometrial adenocarcinoma, FIGO grade 2, with mixed endometrioid, serous and mucinous features and metastases to the left ovary, omentum and lymph nodes. Peritoneal dissemination likely occurred by retrograde transmission via the fallopian tube. Both ovaries were small. Molecular analysis using PCR for the amelogenin locus on the Y chromosome confirmed the presence of the Y-bearing cell line in frozen tissue from the left and right ovaries. Molecular and FISH analyses of additional archived tissues are in process. Normal-appearing ovaries and uterus have been reported in patients with Turner syndrome. Endometrial carcinoma has also been reported both with and without hormone replacement therapy in young Turner syndrome patients. This case and others underline the importance of thorough cytogenetic evaluation for chromosomal mosaicism in Turner syndrome and the increased risk of endometrial carcinoma, as well as gonadoblastoma.

Cytogenetic and FISH analysis of an interstitial 1q42.3-q44 deletion. V. Golembiewski-Ruiz, C.A. Tirado, K. Curesky, J. Goldstein, A. McKonkie-Rosell, M.T. McDonald, B.K. Goodman. Departments of Pathology and Medical Genetics, Duke University Medical Center, Durham, NC.

1q distal deletion syndrome is most often associated with cleft palate, flat nasal bridge, strabismus, hypotonia, severe mental retardation and seizure disorders. We report a 33 month-old girl with a previous karyotype of 46,XX,del(1)(q42.3). Maternal peripheral blood karyotype was normal and a paternal sample was unavailable. An extended family history showed no history of mental retardation, birth defects or translocation. Prenatal ultrasound had shown multiple brain cysts. Delivery was at 37 weeks gestation, and the neonate remained hospitalized for poor feeding and low birth weight. In order to further define the deletion in this patient, we performed FISH analysis using the Vysis chromosome 1 subtelomeric probe set. Both short and long arm loci were intact. To determine whether this was only a deletion or whether material from another chromosome was involved, we performed multicolor FISH (M-FISH) analysis. There was no apparent material derived from any other chromosome. In order to confirm the interstitial nature of the deletion, we used an investigational 1q subtelomeric probe located slightly more proximally in the subtelomeric region of 1q. This locus was also intact. Patients with 1q distal deletions have been reported with brain cysts, midline defects and psychomotor delays. To our knowledge, a subtle interstitial deletion of chromosome 1 bands q42.3-q44 such as that defined in this case has not been reported. A more proximal interstitial deletion, 1q42.1-q42.3, was reported with mild phenotypic effects. Our patient presented with similar features to many of those observed in patients with both terminal and interstitial 1q deletions. These include frontal bossing, cleft palate, flat nasal bridge, hypertelorism, strabismus, severe developmental delay and seizures. A previous brain MRI showed bifrontal atrophy. Our results show that the molecular cytogenetic tools now available will assist in defining deletions such as this and, as in this case, may further localize the critical region for the deletion 1q phenotype.

Interstitial deletion of chromosome 4p in a three-generation family with minimal phenotypic effects. S.L.

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We describe a three-generation family that shows segregation of an interstitial deletion (p14p15.1 or p15.1p15.2) on the short arm of one chromosome 4 homolog but with minimal apparent phenotypic effects. Concern began when the proband was not speaking at age 16-18 months. Formal evaluation demonstrated a delay in expressive language and motor development with hypotonia and a positive Babinski reflex. The child had normal receptive language, hearing, brain MRI, and Fragile X DNA testing. At 44 months, her fine motor testing indicated an age equivalent of 31 months with delays in eye-hand coordination and manual dexterity. The proband's GTG banded karyotype was 46,XX,del(4)(p14p15.1 or p15.1p15.2). The karyotypes of the paternal grandfather, mother, and younger sibling also showed the same 4p interstitial deletion in all cells. The maternal grandfather exhibited normal motor and language development. The mother showed no developmental problems and performed well in college. The proband's younger sister was normal at 24 months in all developmental aspects. To confirm and characterize the deletion, BAC clones mapping to the 4p13-p15.3 region were used for FISH on metaphase spreads from the proband and mother. Based on FISH results and the draft map for human chromosome 4, the deletion size is <5Mb in a relatively gene-poor euchromatic region that is flanked by gene-rich regions. The phenotype associated with this deletion is extremely mild, but may include genes that when haploinsufficient can lead to delay of language and motor functions with variable expressivity. At least 20 individuals have been reported to have 4p interstitial deletions and compared with our family, these individuals have more serious phenotypic effects, which are likely due to different deletion breakpoints involving additional chromosome segments and genes.

Subtelomeric and locus specific FISH as adjunct to conventional cytogenetics elucidate chromosome rearrangement in a de novo complex variant of Wolf-Hirschhorn syndrome. *N.S. Mitter¹, T.T. Brown², G.B. Bromage², M.E. Mangual², J.S. Morales², W.L. Flejter², S.L. Gersen² and Dianon Systems, Inc.* 1) NeoGenomics, Inc., Naples, FL; 2) Dianon Systems, Inc., Stratford, CT.

Wolf-Hirschhorn (WHS), a contiguous gene syndrome, is associated with a deletion of a 165kb critical region in chromosome band 4p16.3. Some cases with small deletions have more severe presentations while others with large deletions have mild clinical features. One explanation for this phenomenon could be in the etiology of the deletion. Cryptic unbalanced translocations between 4p and other chromosomes may appear as deletions of 4p, but with a mixture of features associated with both the deleted 4p and the duplicated genetic material. The use of subtelomeric and region/locus specific FISH probes can help elucidate the exact chromosomal rearrangement involved. This was illustrated for a 2-day old female, referred for cytogenetic analysis for a suspicion of trisomy 18. GTW banding revealed a faint G-positive band in the telomeric region of the short arm of one chromosome 4, interpreted as add(4)(p16.3). FISH analysis with probes for the subtelomeric regions of chromosome 4 showed the absence of a signal on one 4p. Analysis with Vysis ToTelVysion revealed a presence of the 8p telomeric region on 4p as well as two normal chromosomes 8. This was confirmed with the use of WCP8 probes. Similar analyses on parents revealed normal karyotypes and FISH signals. The child, therefore, had an unbalanced de novo rearrangement with a monosomy for distal 4p and a trisomy for distal 8p. Subsequent FISH analysis showed a deletion of the WHS critical region on the der(4p). The final karyotype was: 46,XX,add(4)(p16.3).ish der(4)t(4;8)(p16.3;p23)(D4S3359-,D4S2930+,D8S504+,WHSCR-). The clinical phenotype of distal trisomy 8p includes minor facial anomalies, hypotonia and developmental delay. The proband has mild abnormalities seen in both WHS and distal trisomy 8p (orbital hypoplasia, wide anterior fontanel, dysplastic ears, short bulbous nose) and others that are limited to WHS (triangular face, high arched palate, retrognathia, hypotonia). Family was counseled for a low recurrence risk and follow-up evaluations.

Tetrasomy 9q11-q22.1 in an infant with cleft palate and multiple anomalies. *E.W. McPherson^{1,2,4}, K. Neiswanger¹, M.J. Buckley¹, U. Surti^{3,4}.* 1) Cleft Palate Craniofacial Center, Univ of Pittsburgh School of Dental Medicine; 2) Dept of Pediatrics, Univ of Pittsburgh School of Medicine; 3) Dept of Pathology, University of Pittsburgh School of Medicine; 4) Dept Genetics, Magee Womens Hosp, Pittsburgh, PA.

The proband was the product of an uneventful pregnancy and delivery to a 22 yo G2P1-2. At birth, he was in the 3-10% for weight, length, and head circumference and had a complete bilateral cleft of the hard and soft palate. His facial appearance was unusual with retrognathia, short palpebral fissures, beaked nose with short columella, small mouth, and large, low set ears. He was hypertonic, and had arthrogryposis with wrist flexion and ulnar deviation, camptodactyly, single palmar creases, hammer toes of the hallux, overlapping toes, and metatarsus adductus. He suffered several fractures without known trauma. By one year, his hands had improved, and he was beginning to sit, roll, reach for toys, and say mama. His length and weight were normal, but he had mild microcephaly. His family history was unremarkable. Karyotyping revealed tetrasomy for chromosome 9q13-q22.1, due to a triplication on one chromosome 9 with the normal chromosome complement on the other. FISH utilizing whole chromosome paint for chromosome 9 demonstrated that all of the extra chromosomal material was derived from chromosome 9, for a karyotype of 46,XY, trp(9)(q13q22.1). ish trp(9)(q13q22.1)(wcp+).

While there are several reports of trisomy for different segments of 9q, to our knowledge this is the first report of a patient with partial tetrasomy 9q. Our patient shares several phenotypic features with the trisomy 9q cases, including microcephaly, beaked nose, narrow palpebral fissures, micrognathia, cleft palate, hand and foot abnormalities, and developmental delay. The aneuploid region is similar to that in a patient with trisomy 9q13-q22.1 evaluated for MR, behavioral problems and minor dysmorphic features (Luke et al., AJMG 40: 57, 1991). Partial tetrasomy 9q in our patient appears to cause multiple anomalies similar to trisomy 9q with moderate developmental delay.

A rare case of mosaicism for paternal UPD 9 in a dizygotic twin pregnancy. *K.A. Kaiser-Rogers¹, W.P. Robinson⁴, J.F. Knops⁵, D. Vargo², C.A. Livasy³, J. Bailit², K.W. Rao^{1,3}.* 1) Dept Pediatrics; 2) Dept Obstetrics & Gynecology; 3) Dept Pathology & Lab Medicine, Univ North Carolina, Chapel Hill, NC; 4) Dept Medical Genetics, Univ British Columbia, Vancouver, BC, Canada; 5) Genzyme Genetics, Santa Fe, NM.

In this report we describe the first published case of paternal uniparental disomy (UPD) for chromosome 9. Amniocentesis and karyotyping of a dizygotic twin pregnancy were performed because twin "A" had an enlarged, molar appearing placenta. Growth of twin "A" also lagged 1 week behind its normal appearing twin, "B". Although both twins had a 46,XX karyotype, twin "A" appeared to have two cell lines. In one cell line both chromosome 9 homologues contained a large heterochromatic (qh) region (LL), while 1 large and 1 small heterochromatic region (LS) were seen in the other cell line. Both chromosome 9 homologues within twin "B" had a small heterochromatic region (SS). While both the "SL" and the "SS" cell lines could be explained by normal Mendelian segregation of the paternal "LS" and maternal "SS" homologues, the "LL" cell line in twin "A" could not.

As the pregnancy progressed, twin "A's" growth (22nd centile) continued to lag behind that of twin "B" (58th centile), and at 28 weeks gestation liver cysts were seen. Following the in utero death of twin "A" at 34 weeks gestation, twin "B" was delivered and is currently alive and well. Cytogenetic and molecular studies performed after delivery on the placenta of twin "A", were consistent with mosaicism for a cell line (LL) with complete paternal uniparental isodisomy (UPiD) for chromosome 9. The results obtained from the newborn cord blood were inconclusive. In addition to being the first report of paternal UPD9, this case also represents a rare example of mosaicism for euploid cell lines with UPD and normal biparental inheritance. We speculate that paternal UPD9, especially when present in nonmosaic form, may be lethal in utero. Alternatively, homozygosity for a recessive mutation caused by UPiD9 may be the basis for the clinical findings in twin "A". Absence of a maternal UPD9 affect suggests the latter may be more likely.

Two Cases of Partial Trisomy 13q with Different De Novo Structural Cytogenetic Anomalies and Their Clinical Manifestation. *I. Llano, M.J Zavaleta, R. Melendez, R. Garcia- Cavazos, M. Aguinaga.* Genetics, Inst Nacional Perinatologia, Mexico, Mexico.

Several partial 13q syndromes have been described in the literature, most of them as the result of an unbalanced translocation inherited from a progenitor. Depending on the point of breakage there are widely variable clinical manifestations, the distal deletions being the most severe. Here we report two unrelated cases of patients with distal trisomy 13q due to de novo structural rearrangements involving chromosome 13. Case 1.- 46,XY,homodic(13)(pter--cenq32::q12-cen-pter) and with molecular cytogenetic techniques: 46,XY,homodic(13).ish(CEP13x2)(WCP13+)(LSIx2)(D135327-). Case 2.- 46,XY,add(15)(p13) and with molecular cytogenetics: 46,XY,add(15)(p11).ish dup(13)(qter-q22)(WCP 13+)(D135327+). With these two cases we remark the importance of the clinical examination and correlation with the cytogenetic studies in every patient and his family in the genetics clinic. Even though we now have more sophisticated molecular tools, it is important not to forget the usefulness of the cytogenetic study in almost all of our dysmorphic patient.

A preliminary study to assess the predictive value of a low-resolution genomic microarray in detecting chromosomal imbalances and DNA copy number polymorphisms. *J. LAPIERRE¹, D. SANLAVILLE¹, J. KANG², P. GOSSET¹, C. OZILOU¹, M. Le LORCH¹, C. TURLEAU¹, M. MOHAMMED², S. ROMANA¹, M. VEKEMANS¹.* 1) Departement de genetique, Hopital Necker-Enfants Malades, PARIS, FRANCE; 2) Spectral Genomics Inc., Houston, TX.

Microarray-based comparative genomic hybridization (array-CGH) has been successfully used in the detection of both acquired and constitutional chromosome imbalances. Since these arrays comprise of discrete large-insert genomic clones such as BACs, they may also be used to detect DNA copy-number polymorphisms across the entire genome, which may otherwise be undetectable by conventional cytogenetic methodologies. We decided to test the capacity of a commercially available genomic microarray in detecting constitutional chromosome imbalances as well as its utility in detecting DNA copy-number polymorphisms across the genome. The array chosen for this study was a first generation microarray comprising of 1003 BAC and PAC clones (Spectral Genomics Inc.). DNA samples from patients with well characterized chromosome aberrations as well as from known normal references were tested in a blinded fashion. In accordance with the protocols established for use with these arrays, between 500ng-1g of Cy5 labeled test DNA was co-hybridized with Cy3 labeled reference DNA (forward reaction). Similarly, an equal amount of Cy3 labeled test DNA was independently co-hybridized with Cy5 labeled reference (reverse reaction). While aberrations for which clone coverage was available were detected, our findings suggest that although these arrays were initially designed to offer on average a 3Mb coverage of the genome, the resolution within particularly the subtelomeric regions is lower than 3Mb. The utility of the dye-reversal approach was highlighted by the analysis of one of the known normal reference samples. Importantly, DNA copy number polymorphisms for a single clone on 7q and 16q were observed in both our normal and abnormal DNA samples. Further studies using appropriate DNA clones are in progress to confirm these findings.

Molecular cytogenetic identification of a chromosome 8 derived supernumerary marker chromosome: a clinical description. *J.J. MacKenzie^{1,2}, K.J. Harrison^{1,2}*. 1) Dept of Pediatrics, Queen's University, Kingston, ON, Canada; 2) Dept of Pathology, Queen's University, Kingston, ON, Canada.

A 7 year old boy with developmental delay, inattention and poor coordination was identified to have a karyotype of 47,XY,+mar[21]/46,XY[20].ish der(8)(wcp8+,TelVysion 8p-). He had a prominent forehead, triangular chin, broad nasal root, everted lower lip, arched palate, prominent ears, and increased plantar creases. Initial cytogenetic analysis of 41 G-banded metaphases identified a small metacentric supernumerary marker chromosome (SMC) in 20/41 cells. C-banding identified a single positive centromere region. Paternal chromosome studies (50 cells) were normal. Since the origin of the SMC could not be discerned from the G-banded analysis, it was established using a panel of whole chromosome paint probes (Chromosome Multiprobe-Octochrome). The marker hybridized positively with the wcp probe specific for chromosome 8 and was confirmed using a single wcp8 probe (Vysis). Comparative genomic hybridization studies indicated a possible gain of 8p and 8q material but this was equivocal. The SMC did not hybridize with the TelVysion 8p telomere specific probe. The karyotype was revised to 46,XY,+mar[21]46,XY[20].ish der(8)(wcp8+,TelVysion 8p-). SMCs are a cytogenetic and clinical dilemma, particularly when the chromosome origin cannot be identified or they present in mosaic form. Some phenotypes have been characterized for SMCs derived from chromosomes 9,12,15,18 and 22. The reported features of the few cases identified with SMC 8 overlap with trisomy 8 mosaicism. This case presents a rare clinical condition and illustrates the use of multiple techniques to determine the chromosome origin of the SMC. Probe availability or low level mosaicism can impede the resolution achieved with FISH and PCR, so additional techniques are essential for detailed evaluation. Currently, SMC outcome generalizations can be made based mode of origin, chromosome origin and overall size, but correlation of specific clinical and cytogenetic data provides more predictable phenotypic information which can be applied to counselling and health management.

True fetal mosaicism for a supernumerary *de novo* ring chromosome. *J.M. Meck¹, A. Wray¹, A. Ghidini¹, B. Gorman¹, P. Vallejo¹, T. Dennis², B. Haddad³.* 1) Dept Obstetrics/Gynecology, Georgetown Univ Hospital, Washington, DC; 2) NHGRI/NIH, Bethesda, MD; 3) Dept. Oncology, Georgetown Univ Med Ctr, Washington, DC.

Assessment of the consequences of a *de novo* marker chromosome found prenatally is among the most difficult challenges that a geneticist must face. The best assessments require a thorough classical and molecular cytogenetic workup and excellent prenatal diagnostic testing. We report here on a case in which a small *de novo* supernumerary ring chromosome with apparent euchromatic material was found in 27% of amniocytes at amniocentesis performed for advanced maternal age. Genetic counseling of the couple included a discussion of possible molecular cytogenetic approaches to chromosome identification which included microdissection, SKY, and standard FISH testing. The ring chromosome was lost in subcultures, making FISH diagnosis difficult. The couple elected to have a targeted 2-D and 3-D sonogram, fetal echocardiogram, fetal blood sampling, and a repeat amniocentesis. Both the sonogram and fetal echo were normal. The ring chromosome was found in 10% of fetal lymphocytes, and in 43% of colonies from the repeat amniocentesis. The marker did not hybridize to FISH probes for SNRPN or the centromeres of chromosomes 15, X or Y. The couple was counseled that the FISH results ruled out the marker as being derived from (1) the chromosome which is the most common source of markers (chromosome 15); and (2) chromosomes for which there is substantial prognostic information (X, Y, and 15). Due to the advanced gestational age (23 weeks), uncertainty as to the significance of the mosaicism and its extent in other tissues, the couple elected to have a pregnancy termination without additional testing (SKY, microdissection). Results from the autopsy as well as further molecular cytogenetic evaluation of the marker will be presented. Cases such as this are important in order to establish a genotype/phenotype correlation for other such diagnostic dilemmas.

Phenotypic diversity associated with idic(15) marker chromosomes. *S. Mann¹, D. Liu¹, N. Wang¹, L. Wang¹, N. Dorrani¹, L. Espana², C. Williams-McGovern², C. Fernandez², A. Lopez-Singh², M. Jimenez², M. Sigman², C. Schanen³.* 1) Dept Human Genetics, UCLA, Los Angeles, CA; 2) Neuropsychiatric Institute, UCLA, Los Angeles, CA; 3) Nemours Research Programs, A.I. duPont Hospital for Children, Wilmington DE.

The most common cytogenetic abnormalities observed in autistic probands are aberrations of chromosome 15, specifically 15q11-q13. These duplications frequently take the form of supernumerary pseudoisodicentric marker chromosomes, idic(15). Although they clearly impart a major risk for autism, the phenotypic consequences of these duplications are remarkably variable, but often include some degree of mental retardation, neurologic symptoms, and facial dysmorphisms.

A cohort of 18 probands with idic(15) chromosomes were clinically assessed for abnormalities commonly associated with idic(15) including measures of cognition, language, play and specific testing for autism. Detailed molecular and cytogenetic analyses were performed to identify the extent of the duplicated material. Parental inheritance and methylation status were also ascertained.

Most of the idic(15) chromosomes are structurally stereotyped arising from exchanges through a series of common breakpoints, which allow the critical portion of 15q to be divided into A, B, C regions. However, several were found to be asymmetric leading to trisomy rather than tetrasomy of the involved segments. Comparison of data from probands whose duplications include only AB versus ABC material indicates that autism, seizures, hypotonia and dysmorphisms occur at similar frequencies in both groups. Surprisingly, one autistic female was found to have a paternally derived duplication. Severe cognitive and language deficits occurred in both groups, with some improvement in language scores with age. Within groups, however, there was marked variability in performance. These data underscore the molecular diversity that likely underlies the difficulties in determining genotype-phenotype correlations in idic(15) probands.

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Mosaic, SNRPN positive, Inversion Duplication 15 without Phenotypic Effect in a Mother and Daughter. *E.D. Wood, A. Patel, K.J. Blakemore, G. Stetten.* Johns Hopkins University, Baltimore, MD.

Inversion duplication of chromosome 15 [inv dup (15)] is the most common supernumerary marker chromosome detected during routine karyotyping. The phenotype ranges from normal to severely mentally retarded, related to size of the marker and presence of the SNRPN region. Case reports of both non-mosaic, SNRPN positive cases and mosaic, SNRPN negative cases have been published. An abnormal phenotype has been associated with the SNRPN positive cases in most reports. There are no previously published cases of individuals mosaic for a SNRPN positive inv dup (15). We report a mosaic SNRPN positive inv dup (15) in an apparently unaffected mother and daughter ascertained through prenatal diagnosis. An amniocentesis was performed in a 30 year old G1P0 for a choroid plexus cyst at 20 weeks. Analysis showed that 6/16 (38%) of the amniocyte colonies contained a supernumerary, bisatellited, pseudodicentric marker chromosome identified as a SNRPN positive inv dup (15). Additional FISH studies revealed the inv dup (15) to be positive for D15S11 and D15S10 region, and negative for GABRB3. The maternal karyotype was also mosaic for the same marker in 6/20 (30%) of peripheral lymphocytes. Grandparental karyotypes were normal. Despite similar findings in an apparently normal parent, concerns regarding fetal outcome persisted due to the possibility of UPD 15, the unpredictable effects of imprinting in this region of chromosome 15, and the association between SNRPN positive inv dup (15) markers and abnormal phenotypes. UPD studies revealed normal, biparental inheritance. While most de novo inv dup (15) markers in the literature are of maternal origin, the origin of the de novo marker in the mother is still under investigation. The mother is phenotypically normal with no history of developmental delay. Her daughter, now 17 months old, is non-dysmorphic and showing normal development.

Familial translocation with partial trisomy of 9q34 and 22q11: an expansion of the genomic disorders of 22q11.

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Region 22q11 is a model for disorders caused by chromosomal rearrangements involving large regions (Lupski, 1998) because it is involved in a number of relatively common chromosome disorders including VCFS/DGS, CES, and the der(22) syndrome. Nonhomologous recombination followed by meiotic nondisjunction results in the der(22) syndrome. Usually, this involves a nondisjunctional event in a carrier of a constitutional t(11;22) translocation. We have identified a patient with a der(22) syndrome secondary to the malsegregation of a maternal (9;22) translocation. He is a 9 year old son of double first cousins. His mother lost 7 first trimester pregnancies and a male infant who survived only hours. He underwent unsuccessful glaucoma surgery at age 3 and the repair of a VSD at 5 years. His development has been delayed. His physical examination revealed a microcephaly, a synophrys, low anterior hairline and abnormal pinna. He wears a left eye prosthesis and his speech is often unintelligible. An abnormal peripheral leucocyte karyotype was recorded: 47,XY,+der(22)t(9;22)(q34.3;q11.2)mat.ish +der(22)t(9;22)(q34.1;q11.2)mat(WCP9x2,ABLx2,D9S325x3, D22S75x3,ARSx3). Our patient does not have the somatic features of der(22) resulting from a t(11;22). He does have some features associated with CES; including a congenital heart defect and eye abnormality. He lacks the more typical eye and ear anomalies seen in CES. VCFS/DGS and CES result from homologous recombination between blocks of low-copy repeats (LCR22) and der(22) from nonhomologous recombination between AT-rich palindromes. Non-recurring translocations involving 22q11 have been reported but little is known whether or not they preferentially occur in the same LCR. Molecular analysis of this family will help clarify the importance of LCR22 and AT-rich palindromic sequences.

Inherited mosaic supernumerary marker chromosome (SMC) 8 in a patient with mental retardation: a potential counseling challenge. *M.T. Velinov¹, H. Gu¹, M. Genovese¹, C. Duncan¹, S.S. Brooks², E.C. Jenkins¹*. 1) Cytogenetics, IBRDD, Staten Island, NY; 2) Human Genetics, IBRDD, Staten Island, NY.

A 30-year-old male with mental retardation, long narrow face and high arched palate presented with mosaicism for a small SMC in 90% of his peripheral blood cells. The SMC was shown by multiplex FISH analysis to have originated from chromosome 8. This individual's phenotypically normal mother exhibited the same SMC in 8% of her cells. One additional family with inherited SMC-8 in two affected children has been reported to date (Rothenmund et al. *Am J Med Genet*, 72:339, 1997). In this report one phenotypically normal parent also exhibited low level mosaicism for the marker chromosome 8. These two families illustrate the potential difficulties that may occur in genetic counseling, since at present it is impossible to establish a specific cut off in the mosaicism level for SMC-8 that would result in an abnormal phenotype. The size and chromosomal origin of each SMC is crucial for the effect on phenotype. In addition, inherited SMC's from unaffected and non-mosaic parents typically do not result in abnormal phenotypes. However, families have been reported with poor reproductive history or children with congenital abnormalities/mental retardation in which one of the parents is mosaic for SMC. Accordingly, the reported family supports the concept that phenotypically normal individuals who are mosaic for SMC have higher reproductive risk compared to those with SMC in all cells. Potentially harmful SMC may be "silent" in the parent with low-grade mosaicism, but may have observable phenotypic expression in the fetus/child since it would be present, at least initially, in all fetal cells. We suggest that the inheritance of SMC from a mosaic parent is associated with higher risk for an abnormal phenotype. [This work was supported in part by NYS Office of Mental Retardation and Developmental Disabilities].

Family with three Prader-Willi syndrome siblings carrying a paternally inherited deletion of SNRPN. P.D.

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We describe a family with three Prader-Willi syndrome children; a daughter born in 1977, and two sons born in 1982 and 1984. By age 10, the first son was suspected of having the syndrome due to his clinical history (premature birth, hypotonia, respiratory distress, neurological deficit, epicanthal folds, cryptorchidism, hyperactivity, IQ of 80, small build, small hands and feet, food-seeking behavior by age 3, and increased weight gain). A high-resolution karyotype analysis was performed in 1992, and was normal (no deletion in region 15q11-q13). His sister was also tested at that time, as she had some PWS features (decreased fetal activity, hypotonia at birth, rapid weight gain, small hands and feet, failure to thrive, attention deficit disorder, IQ of 55, and delayed motor development). Her hi-res karyotype was also normal. Additional testing was requested on the first son; In 1994, two FISH probes, GABRB3 and D15S11, were used to detect submicroscopic deletions. The results were negative. In 2001, the first son was re-tested by both FISH SNRPN probe and methylation tests. These tests were positive. The second son (with a history of cryptorchidism, speech delay, hyperactivity and attention deficit, but negative for decreased fetal activity and hypotonia) was also tested by FISH, and was also positive for a SNRPN deletion. Subsequent testing of family members included the sister and the father of the three children, both testing positive for a SNRPN deletion. The father is asymptomatic, presumably carrying the deletion on his maternal chromosome 15 (as a de novo deletion, as his mother tested negative for the SNRPN deletion). This family is of interest due to the variability in expressivity of the identical deletion, the relatively small size of the deletion (which did not include the D15S10 locus), and the fathers normal phenotype.

FISH variants with D15Z1 in clinical cases. *S.H. Shim*¹, *A. Pan*¹, *X.L. Huang*¹, *V.S. Tonk*², *H.E. Wyandt*¹. 1) Center for Human Genetics, Boston University School of Medicine, Boston, MA; 2) Department of Pathology, Texas Tech University Health Sciences Center, Lubbock, TX.

Interphase FISH analysis using alpha-satellite probes for centromere identification has been suspect because of occasional heteromorphism and because of shared sequences, especially between chromosomes 13 and 21 and between chromosomes 14 and 22. Classical satellite III (D15Z1) in the short arm of chromosome 15 reportedly also cross-hybridizes with other acrocentric chromosomes about 10% of the time. We present our experience with cross-hybridization of D15Z1 in clinical cases referred for Angelman (AS) or Prader-Willi syndromes, for autism to rule out duplication of 15q12, or to identify a supernumerary marker chromosome. D15Z1 is commercially available in dual-color probe mixtures in combination with D15S10, D15S11 or SNRPN (Vysis, Downers Grove, IL). Of 38 clinical cases studied with at least one of these probe mixtures, 9 showed an extra signal with D15Z1 on a chromosome 14 and one case showed absence of the D15Z1 signal from 15p. Three of the nine cases with an extra signal on chromosome 14 have additional chromosome abnormalities, including two with a supernumerary marker derived from 15 and one case with der(Y)t(Y;15). Two sibs referred for possible AS show an extra signal on a chromosome 14 in one sib but not in the other. The overall frequency of 23.7% with a signal on chromosome 14 and the high percentage of positive cases (33.3%) with structural abnormalities suggest an unusual correlation with the presence of the D15Z1 signal on chromosome 14. However, in one case with a maternally derived idic(15), the variant 14 was paternal in origin. The absence of the variant 14 in one of the two sibs referred for AS, also make it unlikely the D15Z1 variants have any causative role in any of the clinical or structural chromosome abnormalities studied.

Preeclampsia and confined placental mosaicism. *P.J. Yong, S. Langlois, P. von Dadelszen, I.J. Barrett, D.K. Kalousek, W.P. Robinson.* Depts of Medical Genetics, Obstetrics, and Pathology, UBC, Vancouver, BC.

Confined placental mosaicism (CPM) is present in ~2% of pregnancies and may contribute to the etiology of preeclampsia in cases where the fetus appears karyotypically normal. We sought to determine whether there was an association between preeclampsia and CPM of trisomy 16 (CPM16). Data were collected from a sample of prenatally diagnosed CPM16 pregnancies that continued past 20 weeks, consisting of 6 cases diagnosed in Vancouver and 30 cases from other centres. The clinical definition of preeclampsia from the Australasian Society for the Study of Hypertension in Pregnancy (2000) was used. The prevalence of preeclampsia was 14% (5/36). In the general population, preeclampsia is cited to occur in 3-5% of pregnancies, which was confirmed by control data from BC Womens Hospital. Using 5% as the background prevalence, the relative risk (RR) for preeclampsia in CPM16 was 2.70 ($p = 0.04$). This is a conservative estimate, however, since obstetric data were missing for some cases. Considering the 14 cases where an explicit statement was made about the presence or absence of preeclampsia, the prevalence was 36% (5/14) ($RR = 7.14$, $p < 0.01$). Since poor trophoblast invasion is associated with preeclampsia, the level of trisomy in trophoblast isolated from term placenta was compared between the 5 cases with preeclampsia and the 31 other cases. The cases with preeclampsia had a higher level of trisomy in the trophoblast: 86% +/- 9% vs 55% +/- 9% (mean +/- SE, $p = 0.05$). The preeclampsia cases also had a higher frequency of males (80% vs 21%, $p = 0.02$), and a trend towards higher frequency of maternal uniparental disomy of chromosome 16 (upd(16)mat) (60% vs 34%, $p = 0.27$). There were no significant differences in clinical outcome, except for a higher risk of hypospadias among the preeclampsia cases (100% vs 17%, $p = 0.05$). This supports previous reports of an association between hypospadias and preeclampsia. Our results suggest that trisomy 16 trophoblast may have a poor invasive phenotype. Furthermore, trophoblast that are XY and/or upd(16)mat may have reduced proliferation, increasing the proportion of trisomic trophoblast, and thus, the risk of preeclampsia.

A molecular cytogenetics study of 21 patients with Neonatal Diabetes Mellitus. *C. Borie¹, O. Dupuy¹, D. Genevieve¹, N. Bondeux¹, N. Collot¹, S.P. Romana², M. Polak³, P. Eydoux¹.* 1) Biologie du Developpement, Hospital Robert Debre, Paris, France; 2) Cytogenetics, Hospital Necker-Enfants-Malades, Paris, France; 3) Endocrinology, Hospital Robert Debre, Paris, France.

Neonatal Diabetes Mellitus (NDM), a hyperglycemia appearing within the first month of life and requiring insulin therapy, is a rare disease (1/400,000 live births). Features of this disease include intra-uterine growth restriction, macroglossia, umbilical or inguinal hernia. In half of the cases, neonatal diabetes is transient, disappearing within 6 months. In these patients, there is a predisposition to insulin resistant diabetes. Duplication and inverted duplications within chromosome bands 6q22-23 have been demonstrated in association with NDM; paternal isodisomy of the same region has been shown in other patients. PLAGL1, an imprinted gene located in this region is a candidate gene for NDM. We have tested 21 patients with NDM using conventional cytogenetics and FISH with a probe encompassing PLAGL1. A chromosome rearrangement was barely visible on metaphase chromosomes, but interphase FISH clearly showed a duplicated signal on interphase chromosomes on patients with a duplication demonstrated at the molecular level. The results of this study will be presented. We conclude that interphase FISH is a simple test to diagnose NDM. It may be used in association with molecular diagnosis.

Prenatal Diagnosis by nuc ISH: Patient selection criteria and an analysis of findings. *S.A. Farrell, M.D. Speevak, J. Wilkins.* Dept Lab Medicine, Credit Valley Hosp, Mississauga, ON, Canada.

Nuclear fluorescence in situ hybridization (nuc ISH) now is a commonly used technique for the rapid detection of fetal aneuploidies in amniotic fluid cells. However, the expense, technical time and equipment consumption is high. To conserve laboratory resources, we developed a strategy for selection of patients who could benefit most from this procedure. The Genetics clinic at The Credit Valley Hospital established the following criteria for the selection of patients for nuc ISH. Criteria 1: Late gestation (>21 weeks) and late maternal age (LMA) or screen positive Maternal Serum Screen (MSS) result and late gestation. Criteria 2: A risk of 3% or greater for a chromosome abnormality based on LMA or MSS. Criteria 3: Ultrasound findings highly suggestive of a chromosome abnormality or 2 ultrasound soft signs and late gestation. Over a six month period, nuc ISH was performed on amniotic fluid specimens from 49 patients, representing 8% of the total amniotic fluids tested during that period. Nine (18.4%) patients qualified under category 1; 30 (61.2%) patients qualified under category 2 and 10 (20.4%) patients qualified under category 3. The abnormal nuc ISH findings were: 0/9; 5/30 (16.7%) and 4/10 (40%) respectively. A 100% detection rate of chromosome abnormalities supported the cut-off risk of 3% as compared to a detection rate of 37.5% with a 5% risk cut-off. This analysis justifies the appropriateness of the selected risk criteria. The use of strict selection criteria is a valuable tool, ensuring consistency, fairness, and increased predictability of impending abnormal results.

Detection of chromosomal rearrangements with single copy FISH probe arrays. *J.H.M. Knoll, P. Angell, P.K. Rogan.* Children's Mercy Hospital & Clinics, Kansas City, MO 64108.

Single copy FISH (scFISH; Rogan et al. *Genome Res* 11:1086, 2001) is a technique that quickly generates probes for chromosomal in situ hybridization to sequence-defined genomic intervals. DNA fragments suitable as hybridization probes are generated by long PCR amplification of single copy sequences deduced from the human genome draft sequence. A nick-translated 2 kb fragment is adequately and reliably visualized by indirect immunolabeling. Intervals of at least this length are separated in the genome by an average of 20-30 kb. We have designed and hybridized probes from 21 disease intervals on 15 different chromosomes (Knoll et al. *AJHG* 67S:157, 2000; Rogan et al. *EJHG* 9: 95, 2001). Chromosome breakpoints and gene disruptions in acquired and inherited disorders can be detected and refined by scFISH at a resolution comparable to Southern hybridization and fiber-FISH, neither of which preserve higher-order chromosome structure. We delineate breakage intervals by pooling various combinations of probes derived from an ordered array of single copy sequences adjacent to the breakpoint. We have designed and produced a set of scFISH probes to delineate chromosome 9 breakage intervals in 9;22 translocation-positive chronic myelogenous leukemia, as large deletions proximal of *ABL1* are often associated with a poor prognosis. To increase the throughput of probe synthesis for other chromosomal regions, probe design was automated by developing software to select primers for amplification of scFISH products based on the coordinates of disease intervals in the genome draft assembly. Long PCR reaction conditions were also optimized for preparation and amplification of multiple probes in parallel (96-well format), and amplicons were purified either by reamplification of gel-separated products or by preparative HPLC. These streamlined procedures were used to concurrently design and amplify probes for 24 distinct subtelomeric regions. These probes were selected based on their proximity to chromosomal ends, and therefore should detect subtle, terminal unbalanced rearrangements.

High rate of non-informative FISH patterns is a significant limiting factor in pre-implantation genetics. *R.R. Higgins, J. Ahmad, W. Wyatt.* Allina Cytogenetics Laboratory, Abbott Northwestern Hospital, Minneapolis, MN.

Pre-implantation genetic testing utilizing FISH is performed on blastomeres and sperm to provide genetic information to couples with known balanced chromosome abnormalities. Two examples demonstrate significant FISH signal artifact, due to technical difficulties in processing and random overlap of same color signals, is inherent and prevalent in as high as 50% of our results. Example 1: a couple with the male carrying an $\text{inv}(10)(\text{p}13\text{q}26.1)$. Commercial 10p-arm (green) and 10q-arm (red) sub-telomere probes in conjunction with a centromere 10 (aqua) probe were utilized. The signal pattern for normal/balanced inversion nuclei in sperm was 1 red/1 green/1 aqua (1R1G1A) and in blastomeres was (2R2G2A). The signal patterns for the recombinant $\text{rec}(10)\text{dup}(10\text{p})\text{inv}(10)(\text{p}13\text{q}26.1)$ was in sperm (0R2G1A) and in blastomeres (1R3G2A). The signal patterns for the recombinant $\text{rec}(10)\text{dup}(10\text{q})\text{inv}(10)(\text{p}13\text{q}26.1)$ was in sperm (2R0G1A) and in blastomeres (3R1G2A). FISH was performed on 200 sperm: 67.5% displayed a normal/balanced inversion result, 23.0% displayed a recombinant result and 9.5% were non-informative. 10 blastomeres from 2 cycles of stimulation were studied: 40% displayed a normal/balanced inversion result, 10% displayed a recombinant result and 50% were non-informative. Example 2: a couple with the male carrying a $\text{t}(4;8)(\text{p}14;\text{q}24.13)$. Commercial 4p-arm (green) and 8q-arm (red) sub-telomere probes in conjunction with a centromere 4 (aqua) probe were utilized. A signal pattern for normal/balanced translocations was in sperm (1R1G1A) and in blastomeres (2R2G2A). The 16 signal patterns for the disjunction products were determined. FISH was performed on 200 sperm: 34.5% displayed a normal/balanced translocation result, 65.5% displayed an abnormal result and 0.0% was non-informative. FISH was performed on 10 blastomeres from 1 cycle of stimulation: 40% displayed a normal/balanced inversion result, 30% displayed an abnormal result and 30% were non-informative. A high rate of non-informative patterns is a significant limiting factor in pre-implantation genetics and a significant number of blastomeres are needed to obtain enough informative blastomeres for implantation.

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Submicroscopic deletion 9(q34.4) and duplication 19(p13.3) identified by subtelomere specific FISH probes. *D.I. Quigley, K. Kaiser-Rogers, A.S. Aylsworth, K.W. Rao.* Depts of Pediatrics and Genetics, University of North Carolina, Chapel Hill, NC.

Submicroscopic rearrangements involving chromosome ends are responsible for a number of cases of previously unexplained mental retardation and multiple congenital anomalies. We have studied a patient with mental retardation, significant microcephaly, alopecia totalis and other anomalies who carries an unbalanced product of a cryptic reciprocal translocation involving chromosomes 9 and 19. FISH studies using subtelomere specific probes revealed a derivative chromosome 9 that is missing 9q subtelomeric sequences that are replaced by 19p subtelomeric sequences. The patient has partial monosomy 9q and partial trisomy 19p. The patient inherited the derivative 9 from his father, who carries an apparently balanced, cryptic, reciprocal translocation involving the terminal regions of 9q and 19p. To our knowledge, this is the first report of a patient with the two very rare chromosomal rearrangements, partial monosomy 9q and partial trisomy 19p. This case demonstrates the utility of subtelomere specific FISH probes for detecting cryptic subtelomeric rearrangements in patients with idiopathic mental retardation and normal appearing karyotypes.

Deletion analysis of Smith-Magenis syndrome patients toward further delineation of the SMS critical interval.

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Smith-Magenis syndrome (SMS) is a congenital anomalies/mental retardation disorder and possible contiguous gene deletion syndrome associated with an interstitial deletion of chromosome 17p11.2. The SMS phenotype includes mental retardation, developmental delay, distinct facial anomalies, sleep disturbance, and behavioral abnormalities. The typical deletion carried by >90% of SMS patients is ~4-5 Mb. Using patients with various deletions spanning chromosome 17p; the SMS critical interval is currently narrowed to ~1.0-1.5 Mb of DNA, and contains ~30 known genes and ESTs. To date there has not been a gene identified that is responsible for any of the major characteristics seen in the SMS phenotype, though the gene(s) causing the phenotype are thought to map to this critical interval. In order to further narrow the SMS critical region and determine possible genotype:phenotype correlations, we are searching for patients carrying smaller or abnormal deletions involving 17p11.2. Using fluorescent *in situ* hybridization (FISH), we have completed analysis of 15 patients for deletion of chromosome 17p. Of the 15 patients analyzed, three were found to carry novel deletions involving 17p. Two patients were found to carry larger than average SMS deletions, while the third patient carries an abnormally sized deletion with a breakpoint located within the current SMS critical region. This patient has typical SMS features, and this deletion allows for further refinement of the distal end of the SMS critical interval. Refinements such as this will help to reduce the large number of positional candidate genes currently being studied. Further studies are underway to determine the role, if any, the positional candidate genes may play in the SMS phenotype. In addition, studying patients with these slightly different deletions along 17p may help to explain some of the subtle differences seen in the SMS phenotype.

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Familial variation in alpha satellite regions of chromosomes 18, X and Y can result in inconclusive or false prenatal interphase FISH results. *E.J.T. Winsor, E.M. Wood-Burgess.* Pathology and Laboratory Medicine, Mount Sinai Hosp, University of Toronto, Toronto, ON, Canada.

Commercially available FISH probes are widely used for rapid prenatal diagnosis of common trisomies. In a study of 778 patients using AneuVysion(Vysis) probes on uncultured amniotic fluid or CVS, three patients had an inconclusive result because of a small alpha probe signal (two for chromosome 18 and one for X). Variation in the size of chromosome 18 alpha satellite has previously been reported as a reason for false negative results (Tepperberg J et al.2001; Thilaganathan et al.2000; Weremowicz S et al.2001). Similar variations have been reported using alpha satellite probes for the X and Y chromosomes (Tsuchiya K et al.2001; Tepperberg J et al.2001).

Conclusion: Variation in the size of the alpha satellite region of chromosomes 18,X and Y can result in inconclusive,false negative or false positive prenatal interphase FISH results. These inherited variations may be more common than previously recognized and should be considered when prenatal ultrasound findings are consistent with Trisomy 18 or when there are no ultrasound abnormalities and FISH results indicate 45,X. Use of locus specific probes would reduce these limitations.

Genotype-phenotype correlation in patients with NF1 microdeletion syndrome: identification of candidate genes for mental retardation. *P. Riva¹, M. Venturin¹, P. Guarnieri¹, F. Orzan¹, F. Natacci², C. Gervasini¹, P. Colapietro¹, A. Bentivegna¹, M. Stabile³, R. Tenconi⁴, M. Upadhyaya⁵, C. Hernandez⁶, L. Larizza¹.* 1) Dept Biol & Genetics, Univ Milan, Milan, Milan, Italy; 2) Medical Genetics Unit, ICP Milan, Italy; 3) Medical Genetics Service, H Cardarelli, Naples, Italy; 4) Dept Pediatrics, Univ Padua, Italy; 5) Dept of Medical Genetics, Univ of Wales, Cardiff, Wales UK; 6) Molecular Genetic Unit, H Ramon y Cajal, Madrid, Spain.

NF1 microdeletion syndrome is determined by haploinsufficiency of NF1 gene and its flanking regions in 17q11.2. As indicated in previous studies microdeleted patients (pts) show a more severe phenotype than that observed in classical NF1 pts. To address a phenotype-genotype correlation we have undertaken a study aimed at: 1) grouping the pts on the basis of their extra-NF1 clinical signs; 2) searching candidate genes involved in the outcome of severe phenotype. As NF1 microdeleted pts account for 5-8% of NF1 mutations, we combined the clinical and genetic evidence from 15 of our pts with that concerning 77 similar published cases to evaluate the frequency of each parameter versus the classical phenotype. By means of statistical analysis we found that the most prevalent extra-NF1 clinical signs are mental retardation (MR), heart disease (HD) and dysmorphism. Conventional and fiber FISH analysis allowed our pts to be grouped on the basis of deletion type: REP (1.5 Mb del) and BL (del > 1.5 Mb) which distal and proximal boundaries were marked by 271K11-474K4 and 252O24-1J8 respectively. By using bioinformatics we established the deletion gene content: out of 23 genes, one or several of the following genes, CREME9, CENTA2, OMG, EVI2A, KIAA1821, included in both deletion types and of the five genes in the BL deletion, CDK5R1, ZNF207, NJMU-R1, ACCN1, MYO1D, might be implicated in MR. In particular CDK5R1, involved in the development of cerebral cortex, is currently processed for mutation analysis on pts with non X-linked unspecific MR. Only the JJAZ gene appears a suitable candidate for HD; additional genes with unknown function might be identified by expression and bioinformatic studies.

Genomewide screening with automated fluorescent genotyping to detect cryptic cytogenetic abnormalities in children with idiopathic mental retardation. *M. Rio¹, V. Cormier-Daire¹, S. Lyonnet¹, J. Amiel¹, D. Sanlaville¹, D. Bacq², M. Vekemans¹, A. Munnich¹, L. Colleaux¹.* 1) INSERM U393, et Departement de Genetique Hopital Necker-Enfants Malades, Paris, France; 2) Centre National de Genotypage, Evry, France.

Mental retardation (MR) is the most common developmental disability, affecting largely 2% of the general population. However, its origin remains poorly understood. The causes of MR are diverse, but chromosomal rearrangements are believed to account for 4 - 28% of cases. Because no practical way for screening the entire genome is available at present, efforts have first focused on rearrangements involving subtelomeric regions. We previously developed an efficient strategy based upon automated fluorescent genotyping to test for telomere integrity. This strategy detected about 9 % of cryptic subtelomeric deletions or duplications and 1.4% of uniparental disomies in patients with idiopathic syndromic MR.

Since telomere screening is a first step towards the goal of analyzing the entire genome for chromosomal rearrangements in MR, we decided to extend our strategy to 400 markers evenly distributed along the chromosomes to detect interstitial anomalies. Similarly to telomeric genotyping, the genotype of the affected child was compared to the parental genotypes seeking for irregular allele inheritance. A total of 100 individuals were tested. Results for 45 children have already been analysed and 7 interstitial anomalies were found : 6 deletions and 1 parental disomy. Our data demonstrate that fluorescent genotyping is a sensitive and cost-effective method that not only detects small interstitial rearrangements but also provides a unique opportunity to detect uniparental disomies.

This study emphasizes the value of fluorescent genotyping as a systematic screening approach for the detection of interstitial aberrations. In addition to its clinical relevance, this study will hopefully allow the delineation of new contiguous gene syndromes and the identification of new imprinted regions.

Chromosomal Analysis, Y-microdeletion Detection and their Implications on Subfertile ICSI Men Candidates.

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One hundred and thirty two subfertile candidate men for ICSI treatment were subjected to chromosomal analysis and Y chromosome microdeletion detection prior to ICSI. Sex chromosome aberrations in pure and mosaic forms were diagnosed in 6 cases (4.5%). Y- microdeletion detection in the AZFc region was detected in 3/126 males (2.4%). Fertilization and implantation rates were significantly reduced in subfertile men with AZFc microdeletion ($P < 0.05$) when compared to those with no deletion. As Y- deletion is genetically transmitted from subfertile men to their male offspring via ICSI, genetic counseling and reproductive options have to be offered to those men before ICSI treatment. Preimplantation genetic diagnosis and female sex selection may present another strategy for infertile men with Y- microdeletion to avoid fathering a son with the same fertility problem.

Preimplantation Genetic Diagnosis (PGD) for translocation carriers. *S.W. Cheung¹, W.S. Wun², M.L. Cooper¹, S.C. Angus², A.A. Ybarra¹, J.R. Nichols¹, G.M. Grunert².* 1) Dynagene, Houston, TX; 2) Obstetrical and Gynecological Associates, Houston, TX.

Preimplantation Genetic Diagnosis (PGD) was used for translocation carriers at risk of transmitting chromosome abnormalities to offer the possibility of selectively transferring unaffected embryos. We report three cases in which one spouse is a reciprocal translocation carrier. They are [1] t(3;15)(q26.2;q22.3), [2] t(4;13)(q34;q12) and [3] t(3;15)(q29;p13). Fluorescent in situ hybridization (FISH) for the detection of chromosome abnormalities was utilized prior to embryo transfer. Following In Vitro Fertilization, embryo biopsy was performed on all embryos with 5 cells or greater on day 3. All blastomeres were subjected to two rounds of multicolor FISH for chromosomes 13, 16, 18, 21, 22, X and Y (Vysis) followed by a third round of multicolor FISH for the detection of unbalanced translocations. A three-color scheme consisting of one telomeric probe each for the short and long arm of one translocation chromosome (labeled with Spectrum Green and Orange respectively from Vysis) and a third telomeric probe for one arm of the second translocation chromosome (Texas Red from CytoCell) were used in all cases. This approach enables us to distinguish the balanced signal pattern from that of the unbalanced signal pattern simultaneously in a given blastomere. Therefore it allows for detection of all unbalanced products of meiotic segregation. Only one cell was biopsied and only normal or balanced embryos were transferred. All patients underwent genetic counseling to discuss the risks and benefits of the testing prior to the PGD procedure. A total of 39 embryos in 3 cycles were biopsied. Following FISH analysis, 3 embryos were undiagnosed. Of the 36 embryos that were diagnosed, 10 embryos (27.7%) had a normal complement of the chromosomes tested, and 26 (72.3%) were determined to be chromosomally abnormal (numerical and structural). Three transfers were performed, resulting in one live birth and one ongoing pregnancy. Detailed analysis of the 36 biopsied, normally developing embryos obtained from the three reciprocal translocation carriers will be presented.

Custom Probes for PGD of Acrocentric Chromosome Rearrangements: Use of the BAC Resource Consortium.

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Interphase FISH analysis can be used to detect unbalanced products of meiotic segregation in reciprocal translocation carriers. In such investigations, two probes that flank (or one probe that spans) one of the translocation breakpoints must be employed to detect all unbalanced products resulting from 2:2 segregation. Reciprocal translocations involving acrocentric chromosomes present special problems for probe selection because of extensive DNA homology among acrocentric chromosomes in both the short arm and centromere sequences. We studied a translocation, t(14;21) (q12;q21), present in the male partner of a couple interested in PGD, that required probe development. As both chromosomes involved in the translocation are acrocentric, unique probes were unavailable for the short arm and centromeric sequences. Furthermore, because of the proximity of each breakpoint to its respective centromere, no locus-specific probe mapping proximally to the breakpoint on either long arm was commercially available. The BAC Resource Consortium provides a vast repertoire of FISH-verified human BACs that can be employed for probe development. One of these clones mapping in the region on chromosome 21q proximal to the breakpoint was selected for probe development and used in combination with the Vysis LSI 21 probe mapping distally to the translocation breakpoint. Hybridization to metaphase chromosomes in the translocation carrier verified that the BAC probe mapped proximally to the breakpoint on 21q. A spontaneous pregnancy precluded PGD, but use of these probes permitted the option of CVS for early chromosome analysis, which otherwise would not have been recommended because of the subtlety of this translocation. As illustrated by this case, the extensive collection of clones from the BAC Consortium provides a powerful resource for probe development in FISH diagnostic strategies for both PGD and prenatal diagnosis more generally.

Molecular characterization, IQ and executive functioning in a sample of Turner syndrome cases from Australia.

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We collected a sample of 104 cases of Turner syndrome (TS) for replication of previously reported findings suggesting imprinted, X-inactivation escaping loci affecting behavior on the X chromosome. For 73 cases where DNA was available, we characterized the X chromosome structure, and monosomic or disomic status using a panel of 12 evenly spaced microsatellite markers. 38 cases were monosomic; 24 were maternal in origin (45,Xm), 7 were paternal in origin (45,Xp) and 7 could not be determined because parental samples were not available. 35 cases were disomic. 8 had p-arm and 2 q-arm deletions on the paternal chromosome, and 10 had p-arm deletions on the maternal chromosome. 4 p-arm and 1 q-arm deletions could not be assigned parental origin. 10 cases show a complex pattern and are being further characterized. IQ results were in the normal range (Mean 101.16, SD 12.61) and cases did not differ from controls for either verbal or performance measures. In general, cases performed significantly less well on tests of executive functioning compared to controls. Specifically, selective attention and ability to sustain attention was impaired. Cases showed deficits on the contingency naming task (CNT) and controlled oral word association test (COWAT). When compared to the normative sample TS girls performed significantly less well on accuracy of copy, recall and organisational ability. The pattern of performance exhibited by the (45,Xp) and (45,Xm) were very similar both on measures of IQ and executive functioning. These findings are not in accordance with previous reports, suggesting that (45,Xp) females cannot be distinguished from (45,Xm) females by their performance on these tests.

Trisomy 15q11.2-qter resulting from unbalanced translocation t(X;15)(q22.3;q11.2) in a phenotypically normal girl.

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Only a few unbalanced X;autosome translocations associated with a normal phenotype are known. We report a twin girl with minor growth retardation but no other phenotypic features, in whom a nearly complete trisomy 15 due to a *de novo* unbalanced translocation t(X;15) was identified prenatally (nuchal translucency). Karyotyping, M-FISH and multicolour banding revealed an unbalanced karyotype 46,X,der(X)t(X;15)(q22;q11.2) in the female twin and a normal karyotype in the male twin. At 1 year the girl's development does not differ significantly from her twin. In all cells, BrdU studies showed late-replication of the der(X) with variable spreading onto the translocated 15q. The paternal origin of the der(X) was verified by the CGG-repeat analysis in *FMRI* and the *AR* methylation assay. The 15q11.2 breakpoint was mapped between cen15 and the PWS/AS region. Microsatellite analysis revealed that the additional 15q material is an identical copy of the paternal chromosome 15. The *SNRPN* locus on the derivative chromosome is unmethylated, although X-inactivation appears to spread onto the translocated autosomal DNA. By FISH, BAC clone RP11-483F6 was found to span the Xq22.3 breakpoint. This BAC contains significantly more LINE1 elements (44.69%) than clones in flanking Xq segments. Recently, Bailey et al. (2000) proposed that L1 elements may serve as DNA signals for X-inactivation propagation along the normal X. We hypothesize that in X;autosome translocations, spreading of X-inactivation may be mediated only from specific regions of the X chromosome. Clusters of specific DNA domains such as LINE1 sequence may serve as target e.g. for histone proteins, which in turn enable *cis*-propagation of the XIST-RNA from X onto the autosome.

Evidence for sequence specificity in the association of XIST RNA with chromatin. *L.L. Hall¹, C.M. Clemson¹, J. McNeil¹, M. Byron¹, K. Wydner², A.F. Smit³, J.B. Lawrence¹*. 1) Cell Biology, U. Mass. Medical School, Worcester, MA; 2) Department of Obstetrics/Diagnostic Genetics, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ; 3) The Institute for Systems Biology, 1441 North 34th Street, Seattle, WA.

Whether XIST RNA is indifferent to the sequence content of the chromosome is fundamental to understanding its mechanism of chromosomal inactivation. Transgenic Xist RNA appears to associate with and inactivate an entire mouse autosome. Here, the relationship of human XIST RNA to autosomal chromatin is investigated in cells from two patients carrying X;autosome translocations in the context of trisomy for the involved autosome, where there is no selection against autosomal inactivation. Since such trisomies are lethal in early development, the normal or mild phenotypes of the X;14 and X;9 trisomic patients demonstrates that the translocated autosomes were inactivated. Surprisingly, our analyses show that in cultured primary fibroblasts from adult patients, XIST RNA does not associate with most of the involved autosomal material, as it does the X-chromatin. The bulk of the translocated autosome is hypoacetylated and later replicating than the normal autosome, supporting that inactivation hallmarks were present beyond the region associated with XIST RNA. However, several observations indicate that the autosomal inactivation is less complete or stable than X inactivation, which our results suggest is due to a deficient interaction with XIST RNA. These findings reveal a fundamental difference in the affinity of XIST RNA for autosomal versus X chromatin. Further evidence for sequence specificity comes from a reproducible banding pattern of the Xist RNA as it detaches from murine mitotic X chromosomes, indicating higher affinity of Xist RNA for early-replicating R-bands enriched for genes and SINE elements. This specificity of XIST/Xist RNA may point to novel sequence motifs involved in chromosome architecture and genomic regulation.

Expression profile of the human inner ear genes and the identification of candidate genes for genetic deafness.

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Despite the progress in the human genome project and the efforts to identify the genes expressed in the mammalian inner ear, our knowledge of the genes expressed in the inner ear is very limited. Microarray technology has provided a powerful tool that can be used to study the inner ear gene expression. Oligonucleotide array was used to survey genes expressed in the fetal human inner ear. Sub-tissues (the organ of Corti, the ligament) from the inner ear were collected to evaluate the regional expression patterns. In total ~27,000 genes and ESTs were classified as being expressed in at least one of the samples, representing 43% of ~62,000 genes and ESTs surveyed. A majority of genes were found to be expressed in all the tissues, with a subset of genes showed distinct patterns associated with each sub-tissue. Other human tissues were also studied using the same chip set in order to identify the inner ear enriched genes. The tissues included the human brain, heart, kidney, testis and retina. ~2600 (4% of total number of genes surveyed) genes were identified as the inner ear enriched, being either expressed in the inner ear only, or predominantly expressed in the inner ear. Gene ontology representation showed many enriched genes encode for signal transducers, enzymes and transporters. Of 39 deafness genes on the chip 41% came from the inner ear enriched genes, indicating that other genes within the group are more likely to be involved in many forms of deafness. We have mapped all the inner ear enriched genes and their mouse orthologs to the human and mouse chromosomal locations, respectively. This approach will result in the enrichment of candidate genes for deafness by as much as 10 fold. In addition our work also identified genes enriched in other human tissues, thereby providing candidate genes for genetic disorders involving those tissues.

Identification of novel genes involved in the regeneration of inner ear hair cells. *M.G. de Silva*¹, *M. Hildebrand*^{1, 2}, *J. Aloe*¹, *T. Klockars*¹, *H.-H. Dahl*^{1,3}. 1) Murdoch Childrens Research Institute, Royal Children's Hospital, Melbourne, Victoria, Australia; 2) Department of Biochemistry, University of Melbourne, Melbourne, Victoria, Australia; 3) Department of Paediatrics, University of Melbourne, Melbourne, Victoria, Australia.

Hearing loss is the most common sensory condition in our population often resulting from inner ear hair cell degeneration due to genetic abnormalities or environmental insults. In the bird, hair cells can regenerate resulting in restored hearing function. In the mature mammalian inner ear however, hair cells are not replaced once damage has occurred leading to permanent hearing deficits. Our aim therefore, is to identify factors required for hair cell regeneration. Microarrays offer a tool for sensitive, quantitative and efficient analysis of expression changes in thousands of genes in the one experiment. Our microarray analyses of the Soares NMIE library, consisting of 1536 cDNA clones isolated from the mouse inner ear have successfully identified several novel inner ear specific transcripts. In addition we have validated the technology by detecting transcripts that are known to be important in inner ear function such as Collagen II, alpha 1, Collagen I, alpha 2 and Otoconin 90. We are applying this technology to identify factors vital to the process of differentiating an ES cell to an inner ear hair cell. Several growth culture conditions, exposure to EGF, BDNF, NT-3, aFGF, bFGF and conditioned media from cochlea cell explants, have been selected for the examination of gene expression variations as the ES cell population moves along the differentiation pathway. RNA isolated from differentiating cells has been screened by RT-PCR for the expression of the hair cell markers Brn3.1, and myosin vi and viia. In addition the RNA was reverse transcribed incorporating the fluorescent dyes Cy3 or Cy5. The parental ES cell line is used as a reference base and was labelled with the alternate dye prior to co-hybridisation. Using the Soares NMIE library array we have been able to follow expression changes in inner ear-specific transcripts and select genes with interesting expression profiles for further examination.

The Role of FOXI1 in Pendred Syndrome. *K.A. Kolln¹, S. Enerback², R.J.H. Smith¹.* 1) Molecular Otolaryngology Research Laboratories, University of Iowa, Iowa City, IA; 2) Medical Genetics, Department of Medical Biochemistry, Goteborg University, Goteborg, Sweden.

Statement of Purpose. Pendred Syndrome (PS) is an autosomal recessive disorder characterized by severe-to-profound sensorineural hearing loss (SNHL), thyroid goiter and temporal bone malformations (dilated vestibular aqueduct (DVA) and Mondini malformation). Mutation screening of the causative gene, SLC26A4, has facilitated diagnosis, however in many individuals with this phenotype no SLC26A4 mutations are found. In other persons, only a single SLC26A4 mutation is detected. These findings suggest genetic heterogeneity and/or epistatic interactions with other genes. Recently, cochlear abnormalities and SNHL were demonstrated in mouse mutants with targeted deletions of a winged helix transcription factor, Foxi1. In situ hybridization studies of Foxi1 showed that this gene is expressed in the endolymphatic duct and sac in a pattern similar to that seen with Slc26a4. Further studies have shown that Slc26a4 expression is absent in Foxi1 ^{-/-} mutants. Based on these observations we hypothesized that mutations in FOXI1 cause PS in humans. Methods Used. We screened a cohort of 133 families with a PS phenotype who were negative for SLC26A4 mutation screening. Summary of Results. We identified eight single nucleotide polymorphisms (SNPs) in FOXI1. Four SNPs (276: G®A; 969: G®C; 1011: G®A; 1041:C®T) are wobble bases, causing no change of amino acids. Four nucleotide changes (674:C®T, 724:C®A, 769:G®C, 797:G®A) identified in three affected persons result in missense mutations (T199I, P216T, R231G, R240Q) in FOXI1. In mutation screening of 153 random controls, P216T was detected in two individuals. While only two changes are conserved across species (T199I and R240Q), all cause significant alteration in size and polarity of the amino acids. Conclusion. Based on these data, we calculate that mutations in FOXI1 are responsible for 10% of the PS genetic load and are one-fifth as common as mutations in SLC26A4. (Supported in part by R01-DC02842 to RJHS).

Analysis of the ear and craniofacial region in mouse models of VCFS/DGS. *S. Nowotschin¹, S. Raft², J.S. Arnold¹, J. Liao¹, D.E. Febres¹, T. Van De Water³, B.E. Morrow¹.* 1) Molecular genetics, Albert Einstein College of Med, Bronx, NY; 2) Neuroscience, Albert Einstein College of Med, Bronx, NY; 3) University of Miami, Miami, FL.

Patients with velo-cardio-facial syndrome/DiGeorge syndrome (VCFS/DGS) are characterized by defects in the derivatives of the pharyngeal arches. Included among the major malformations of the first and second arches are facial dysmorphism, velo-pharyngeal insufficiency, chronic otitis media, outer ear malformations and submucous cleft palate. In addition, some have conductive or sensorineural hearing loss as well. Genetic complementation studies revealed that one of the genes hemizygotously deleted on 22q11, termed *Tbx1*, is a strong candidate for cardiovascular defects in mouse models. *Tbx1* is a member of the T-box containing family of transcription factors expressed in the otic vesicle and pharyngeal arches. Pathological studies were performed in *Tbx1* heterozygous and homozygous embryos as well as in bacterial artificial chromosome (BAC) transgenic mice overexpressing human *TBX1*. By otocyst stages of ear development, the otic epithelium (rudiment of the sensory organs and VIIIth cranial neurons) is hypoplastic in homozygous mutants, while the ganglion is enlarged. During otocyst stages, areas of epithelial neurogenic gene expression domains are expanded nearly 2-fold in null animals, suggesting a role for *Tbx1* in regulating the otocyst epithelial/neuronal cell fate decision. BAC transgenic mice had hypoplasia of the sensory organs and chronic otitis media, both found in VCFS/DGS patients. In addition to the ear, *Tbx1* homozygotes and BAC transgenic mice had a cleft palate. To determine the basis of these, we are examining staged embryos by in situ hybridization with molecular probes for the cochlea, vestibular system and ganglion, including *Pax2*, *Hmx2/Hmx3* and *NeuroD*, respectively. We are also investigating the expression pattern of *Col2a1* to study craniofacial development. We believe that these mice serve as a model to understand the molecular basis of ear and craniofacial disorders associated with VCFS/DGS and more common non-syndromic disorders.

Gene expression profiles of regenerating sensory epithelia in the chick inner ear. *R.D. Hawkins¹, S. Bashiardes¹, N. Saccone¹, D. Messina¹, M.E. Warchol², M. Lovett¹*. 1) Washington University, St Louis, MO; 2) Central Institute for the Deaf, St Louis, MO.

Damage to sensory hair cells (HCs) of the inner ear can result in acquired deafness and balance disorders. Mammals cannot replace damaged cochlear HCs, but avian sensory HCs can regenerate if damaged. These new HCs are derived from the underlying supporting cells of the epithelia. Unless such damage occurs, the HCs of the avian cochlea remain in a quiescent state. However, the nearby HCs of the chick utricle are normally in a constant process of regeneration and apoptosis. We are employing cDNA and oligonucleotide microarrays to pinpoint the gene expression changes that occur during this regenerative process and in the process that occurs after sound or drug damage to the cochlea. Our cDNA arrays consist of amplicons for either 400 genes expressed in the inner ear, or for a more random collection of ~8,000 sequence-verified cDNAs. Our custom oligonucleotide array contains 50mers for >1700 transcription factor (TF) genes (comprising all known TFs plus several new TFs). We have derived multiple comparative expression profiles from the few tens of thousands of cells that comprise the (two-cell thick) sensory epithelia of the cochlea and utricle. Among the most significant differences we have observed are increases in SMAD2, beta amyloid, SPARC, TBX2 and KIT expression levels in the proliferating utricle. In the quiescent cochlea BMP4, GATA3, gelsolin, syndecan4, and FOXF1 are upregulated. We have validated many of these observations by a combination of RNA in situ and quantitative real time PCR. We are also generating profiles from sensory epithelia at various timepoints after damage (either in vivo or in vitro). Preliminary data indicate that the TFs FHL1, JUNB, LAF4, and LDOC1 are upregulated early during the regeneration process. Interestingly, several genes that show changes are either known deafness loci or map close to as yet uncloned loci for hearing loss. These findings should prove useful in identifying new markers for the developing and regenerating sensory epithelia, for identifying regenerative pathways and for deriving candidate disease loci.

Fgf-3 and Fgf-10 in mouse inner ear development. *T.J. Wright, S.L. Mansour.* Dept Human Genetics, Univ Utah, Salt Lake City, UT.

Otic development initiates early in development when signals from the hindbrain and mesoderm induce a region of ectoderm to thicken, forming the otic placode. The placode then invaginates and forms a vesicle that undergoes cellular differentiation and morphogenesis, resulting in a mature inner ear. Studies of mice, chick, and zebrafish have implicated several signalling molecules, including fibroblast growth factors (Fgfs) 3 and 10 in these processes.

Mice carrying targeted mutations in *fgf-3* or *fgf-10* have shown that these genes play important roles in inner ear development. In mice that lack *fgf-3*, induction of the placode and formation of the otocyst occur normally, but defects were observed in the induction of the endolymphatic duct and the subsequent morphogenesis of the otocyst as well as in the formation of the otic ganglion. However, the penetrance and expressivity of the mutant phenotype varied, suggesting that other Fgfs and/or their receptors might also play roles in otic development. Indeed, *Fgf-10* is expressed in the developing ear and *Fgf-10* mutants have small inner ears. This hypothesis was also strengthened by the finding that mice homozygous for a targeted disruption of *fgfr2IIIb*, which encodes the preferred receptor for Fgfs-3 and -10, have an ear phenotype similar to, but more severe and penetrant than those of *fgf-3* and *fgf-10* mutants.

To determine the combinatorial roles of these molecules, we have generated mice that lack both Fgf-3 and Fgf-10. Surprisingly, the double mutant embryos lack otic vesicles. These embryos do not show otic expression of the placode marker *Pax-2* and expression of three additional placode markers are mislocalised. Hindbrain patterning as assessed by in situ hybridisation analysis of *Hoxb1* and *kr* expression in rhombomeres 4-6 in the double mutants is normal. In addition, an intermediate otic phenotype is present in embryos with 3 mutant alleles. Further characterisation of otic development and ganglion formation in double mutant embryos and those with three mutant alleles is underway. These results suggest that Fgf signalling plays a direct role in placode specification.

Cluster analysis of gene expression dynamics in differentiating C2C12 myoblasts. *K. Tomczak*^{1,3}, *M. Ramoni*^{2,3}, *D. Sanoudou*^{1,3}, *M. Han*^{1,3}, *A. Kho*^{2,3}, *I.S. Kohane*^{2,3}, *A.H. Beggs*^{1,3}. 1) Genetics Division; 2) Informatics Program; 3) Children's Hospital, Harvard Medical School, Boston, MA.

We used expression profiling to assay gene expression in mouse C2C12 myoblasts induced to differentiate through addition of fusion media (day 0). Triplicate cultures were analyzed on days -2, -1, 0, 2, 4, 6, 8 and 10 for expression patterns of 24,422 probe sets using Affymetrix MG_U74 Av2 and Cv2 microarrays. Fold comparisons and cluster analysis were performed to identify genes with coordinate expression patterns and variable expression levels between proliferating and differentiating cells. Immunofluorescence and western blotting are being used to establish that protein levels of selected genes correlate with mRNA expression measured by the arrays. We used CAGED (<http://genomethods.org/caged>), a Bayesian program for the analysis of time-ordered microarray experiments, to identify 13 unique clusters. Three clusters best met the normality criteria of the standardized residuals and showed the most dramatic changes with time. Cluster 1 contains 8 probe sets whose expression levels decreased with average fold difference of 87. They all represent genes that are expressed in proliferating cultures and are scored "absent" by Affymetrix MAS 5.0 in differentiated/contact-inhibited ones. Conversely, cluster 13 includes 5 mRNA sequences that are found only in fusing cells with 593 fold up-regulation, on average. Four of these highly up-regulated transcripts represent known muscle genes and one is an EST. Cluster 2 contains 90 mRNA sequences that are also up-regulated over the time course. Not surprisingly, 56 are known muscle genes with 21 sarcomeric proteins (e.g. 9 myosins, 6 troponins) and 11 membrane proteins (e.g. 3 sarcoglycans). More interesting are 34 mRNAs not known to be involved in muscle differentiation. Among them are 18 ESTs with no homology to any known genes. These unknown genes represent new differentiation-induced transcripts whose further characterization will allow a better understanding of muscle differentiation. Moreover, our model can be used as a baseline to compare with C2C12 cells expressing various mutant genes involved in myopathic disorders.

Direct Genetic Influence on Brain Sexual Differentiation. *P. Dewing*¹, *T. Shi*¹, *B. Bowling*¹, *S. Horvath*⁴, *E. Vilain*^{1,2,3}. 1) Human Genetics, UCLA, Los Angeles, CA; 2) Dept of Pediatrics, UCLA, Los Angeles, CA; 3) Dept of Urology, UCLA, Los Angeles, CA; 4) Dept of Biostatistics, UCLA, Los Angeles, CA.

The central dogma of sexual differentiation of somatic tissues is based on the primary dependence on testicular secretions by the developing fetus. This concept of hormonal control of sexual differentiation has been applied to brain sexual dimorphisms, where testosterone, directly or via local conversion to estradiol, promotes the induction of masculine patterns of neural and behavioral development, while preventing feminine patterns of differentiation.

A great deal of experimental evidence from several decades of research supports the role of gonadal steroids being solely responsible for inducing brain sexual differentiation. By acting on neural development, testosterone and its metabolites cause male and female brains to develop slightly differently. Here we propose an alternative theory that sexual differences between male and female brains are also the result of direct actions of genes on the induction of sexually dimorphic patterns of neural development. Using microarrays and confirmatory RT-PCR, we have detected at least seven murine genes which show differential expression between male and female developing brains at E10.5, before any gonadal hormone influence. Three of these genes are enhanced in females and four in male. The identification of genes differentially expressed between male and female brains prior to gonadal formation suggests that genetic factors directly influence brain sexual differentiation.

Central apnea in newborn mice with homozygous SLC5A3 gene deletion. *G.T. Berry¹, R.B. Buccafusca¹, S.W. Wu¹, J.R. Ren², L.G. Gonzales¹, P.B. Ballard¹, J.G. Golden¹, J.G. Greer².* 1) The University of Pennsylvania School of Medicine, CHOP, Philadelphia, PA; 2) University of Alberta, Edmonton, Alberta, Canada.

Myo-inositol (Ins) has been long held to play a special role in brain metabolism because both Ins and its polyphosphoinositide derivatives that are important in membrane signaling are exceptionally abundant in nervous tissue. The Na⁺/Ins cotransporter (SLC5A3 or SMIT) can maintain a high millimolar concentration gradient of cellular Ins and is highly expressed in brain and spinal cord of the embryo and fetus, as well as placenta. In order to gain more insight into the role of the Ins in brain metabolism, we generated a homozygous targeted murine SLC5A3 gene deletion model. All SLC5A3 (-/-) animals expire shortly after birth because of hypoventilation at which time whole body Ins levels were reduced by 84%. Necropsy studies were normal. We utilized an in vitro brain stem-spinal cord -diaphragm preparation to examine the inspiratory motor discharge of 18.5 pc fetuses following SLC5A3 (+/-) X SLC5A3 (+/-) matings. Electrophysiological recordings were also obtained from medullary slice preparations, consisting of a putative ventrolateral respiratory rhythm-generating center, the pre-Bötzinger complex (PBC). The screening for potential mutants with an abnormal respiratory motor pattern was double-blinded. A total of 3 SLC5A3 (-/-), 65 (+/-) and 10 (+/+) newborn mice were studied. Diaphragm EMG recordings from only the SLC5A3 (-/-) pups had irregular respiratory rhythm patterns with 3-8 apneas of 15 to 60 second durations per 10-minute period. Direct recordings of brainstem neuronal population discharge within the PBC demonstrated that the irregular rhythms were present in the center, rather than simply reflecting a failure of transmission of inspiratory drive to motoneuron populations. The lethal hypoventilation can be explained on the basis of central apnea due to a deficit in the brain rhythm-generating center, close to or at the PBC. The homozygous SLC5A3 gene deletion model demonstrates the critical importance of Ins in the physiology of the nervous system, as well the essential nature of the SLC5A3 transporter in establishing an Ins concentration gradient in the fetus.

Novel Developmentally Regulated Gene Circuits in Murine Brain Regions: A Template for Understanding Dysfunction and Tumorigenesis. *E.A. Donarum¹, S. Hautaniemi², J. Graff¹, Y. Cheng¹, S. Mousses², O. Kallioniemi², J. Natale¹, D.A. Stephan^{1,2}.* 1) Research Center for Genetic Medicine, Childrens Research Institute, Washington, DC; 2) Cancer Genetics Branch, National Human Genome Research Institute, NIH, Bethesda, MD.

The gross anatomic changes of the developing brain have been well documented, and to a large extent, correlated with specific brain dysfunction. Dysfunction (tumorigenesis as well as cognitive difficulties) without major structural damage is less well understood. Despite the great advancements in biological knowledge, the developmental pathogenetics underlying most brain phenotypes are not known. Here, we investigate the normal developmental gene regulation program in an in vivo murine model using cDNA array expression profiling of 11,264 genes (BMAP clone set). Four murine brain regions were micro-dissected and expression profiled in quadruplicate at high-density time points between birth and maturation. Waves of gene expression correlating with known anatomic/structural changes were identified. We specifically focused on the expression changes that were known to be involved in axonal migration and myelination, and defined a cohort of genes and ESTs that are temporally correlated with these events. A novel SOM analysis tool was applied to assign novel functions to these gene/EST clusters. This analysis provides a template against which to compare expression profiles of different neurologic tumors (glioma, ependymoma, medulloblastoma) and behavioral murine models (neurofibromatosis) of human disease so that we can rapidly identify developmental stage-specific pathologic expression correlates. For more information on the use of cDNA microarrays as a gene expression platform, refer to <http://microarray.cnmcresearch.org>.

Tumor Suppressor PTEN is a Negative Regulator of TrkA Signalling. *S.A. Moussatov¹, J.M. Roberts², M. Sugiultzoglu², D.W. Pfaff¹, M.G. Kaplitt^{1,2}.* 1) Lab Neurobiology/Behavior, Rockefeller Univ, New York, NY; 2) Dept. of Neurosurgery, Weill Medical College of Cornell Univ., New York, NY.

The tumor suppressor PTEN has distinct spatial and temporal patterns of expression in the nervous system. However, its role in regulating neuronal functions is poorly understood. We have overexpressed PTEN in rat pheochromocytoma PC12 cells, a neuronal in vitro model, and found it to inhibit several NGF-mediated effects. While survival was significantly reduced, neurite outgrowth and NGF-induced growth arrest were blocked. We have shown that activation of the MEK/MAPK signaling pathway by NGF, a major pathway that regulates neuronal differentiation in PC12 cells, was hampered by PTEN. Upregulation of AKT and its downstream targets, which are involved in both survival and differentiation, were also significantly reduced. In addition, PTEN overexpression prevented the expected elevation of peripherin mRNA in response to NGF-induced differentiation. This phenomenon is known to be mediated by PLC-gamma signaling, suggesting that PTEN might negatively regulate this pathway as well. In search of the mechanisms of PTEN action on the above cascades, we have discovered that PTEN dramatically inhibits phosphorylation of the kinase domain of the NGF high affinity receptor TrkA at Tyr674/675. Consistent with this observation, the phosphorylation of TrkA at Tyr490, critical for the activation of the MEK/MAPK cascade, was also significantly reduced. To our knowledge, this is the first evidence of negative regulation of multiple signal transduction pathways by PTEN at the level of a neurotrophin membrane receptor. Given the fact that NGF elicits a wide variety of responses in PNS and CNS both during embryogenesis and in adulthood, our findings suggest a role for PTEN in modulating neuronal differentiation, synaptic function, plasticity and survival.

Functional and genetic interactions between Doublecortin and LIS1 in neuronal migration. *T. Tanaka*¹, *C. Paczkowski*¹, *F.F. Serneo*¹, *S. Sasaki*³, *M.J. Gambello*², *S. Hirotsune*³, *A. Wynshaw-Boris*², *J.G. Gleeson*¹. 1) Dept Neurosciences, Univ California, San Diego, La Jolla, CA; 2) Dept Pediatrics and Medicine, Univ California, San Diego, La Jolla, CA; 3) Center for Genome Medical Science, Saitama Medical School, Hidaka, Saitama, Japan.

Neurons destined for the cerebral cortex migrate hundreds of cell-body distances to reach their final destination. Neuronal migration is dependent upon *doublecortin* (*DCX*) and *lissencephaly-1* (*LIS1*), as humans with mutations in either gene display defective migration. Here we show that *Lis1* and *Dcx* function on a common pathway in neuronal migration. We adopted a cerebellar granule cell migration assay system with retroviral transduction of either wild type proteins or fluorescently-tagged proteins to study the effect of gene overexpression and to determine the subcellular localization of these proteins during cell migration in vitro. To study the effect of loss of function of *Lis1*, we utilized neurons from *LIS1* deficient mice. *LIS1* deficient neurons displayed a dose dependent decrease in migration distance. Overexpression of *Lis1* or *Dcx* in wild type neurons resulted in increased neuronal migration distance. *Dcx* overexpression rescues the migration defect in *LIS1* deficient neurons. These results suggest that *LIS1* and *DCX* have dosage-sensitive effects on neuronal migration in both deficiency and overexpression, and genetically interact.

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Mutations in the EGF-CFC genes, *CFC1* and *TDGF1*, cause congenital cardiovascular malformations in humans.

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EGF-CFC genes (*Cfc1*, *Tdgf1*, and others) are expressed during early vertebrate development and are implicated in the establishment of the axial midline and left-right axis formation. Recently, mutations in *CFC1* have been shown to cause laterality defects and transposition of the great arteries (TGA) in humans. In addition, *Tdgf1* was found to be involved in cardiogenesis during murine embryonic stem cell differentiation in culture. Our study consisted of 378 patients with a wide spectrum of cardiac anomalies: TGA, tetralogy of Fallot, truncus arteriosus, interrupted aortic arch, and atrioventricular canal. Screening for mutations in *CFC1* and *TDGF1* were conducted by PCR-dHPLC and each identified variants were subsequently sequenced to identify the nucleotide change. In addition to published mutations in *CFC1*, we have identified a single base pair deletion which was previously described to have loss of function in the zebrafish rescue assay, one splice site mutation, one variant in the Kozak sequence -5' untranslated region, seven missense mutations, two intronic variations in the splicing region, and four SNPs. In *TDGF1* we have identified two missense mutations (one each in EGF and CFC motif) and three SNPs. The identified deletion and missense mutations were not detectable among 200 chromosomes from normal controls. Preliminary data from functional studies and/or predicted protein alterations of some of the identified mutations in *CFC1* and *TDGF1* suggest a loss-of-function mechanism as a cause for cardiovascular malformations in humans. We are now testing the functional effects of previously unstudied variants in three assay systems: a) transfection into NIH 293 cells with a reporter readout, b) functional complementation in zebrafish embryos, and c) splicing analysis by RT-PCR in transfected COS cells.

Role of *FAST1* gene in the development of holoprosencephaly (HPE) and congenital cardiac malformations in humans. *M.V. Ouspenskaia*¹, *J.D. Karkera*¹, *E. Roessler*¹, *M.M. Shen*², *E. Goldmuntz*³, *P. Bowers*⁴, *J. Towbin*⁵, *J. Belmont*⁵, *M. Muenke*¹. 1) MGB, NHGRI/NIH, Bethesda, MD; 2) CABM/UMDNJ-RWJ Medical School, Piscataway, NJ; 3) The Children's Hospital of Philadelphia, Philadelphia, PA; 4) Yale University School of Medicine, New Haven, CT; 5) Baylor College of Medicine, Houston, TX.

Fast-1 (Fox-H1) is a transcriptional factor that in complex with Smad2 and Smad4 mediates TGF- β , Activin and Nodal signaling pathways. These factors control early development in vertebrates and are vital for specification of the anterior-posterior (AP) axis. Recent studies on murine *Fox-H1* knockouts show that embryos failed to pattern the AP axis, form the node, prechordal mesoderm, notochord and definitive endoderm. These mutant mice demonstrate aberrant anterior head structures and abnormal cardiac development. Based on these studies we screened 100 patients with familial holoprosencephaly (HPE) and 350 patients with congenital cardiac anomalies, including tetralogy of Fallot, transposition of the great arteries, double outlet right ventricle, interrupted aortic arch and atrioventricular canal. We characterized the intron-exon structure of human *FAST1* gene. Then using PCR-dHPLC, SNPs carrying variants were identified and sequenced. We have found 20 missense variants, two deletions, one 13 bp intronic insertion and several polymorphic variants. Three of these mutations were seen both in HPE and cardiac patients. None of the described mutations was found in 200 normal chromosomes. These mutations are present in the DNA-binding (Fork head) domain of FAST1 and in the SMAD interaction domain, which is involved in FAST1/SMAD2 interaction. To provide evidence for the predicted loss-of-function, the following functional studies are in progress: a) RNA injection into the Fast-1^{-/-} zebrafish mutant *schmalspur*, b) transfection into 293 cells with reporter read-out, and c) transfection followed by co-immunoprecipitation to assess interactions with SMADs and related co-factors. If confirmed by these studies, *FAST1* will be the first gene shown to cause midline (HPE) and laterality (abnormal cardiac looping) defects in humans.

Heart defects in X-linked heterotaxy: evidence for genetic interaction of *Zic3* with the TGF-beta signaling pathway. *S.M. Ware, K. Harutyunyan, B. Casey, J.W. Belmont.* Molecular & Human Genetics, Baylor College Medicine, Houston, TX.

Heterotaxy, the random assignment of visceral organs with respect to each other and the left-right axis, is characterized by complex congenital heart defects. Mutations in the zinc finger transcription factor *Zic3* cause X-linked heterotaxy, HTX1. Disruption of a critical asymmetric signaling pathway involving the TGF-beta family member *nodal* during early embryogenesis results in heterotaxy in animal models. We have investigated the interaction of *Zic3* and *nodal* in order to understand the role of *Zic3* in cardiac development and left-right axis formation. A targeted deletion in the murine *Zic3* locus has been created and we have previously shown that these knockout mice correctly model HTX1 (Development 129: 2293-2302, 2002). Embryonic lethality occurs in *Zic3* null mice due to both gastrulation defects as well as cardiac defects. In situ hybridization of gastrulation stage embryos indicates defects in migration through the primitive streak. Analyses of null embryos during the looping phase of cardiogenesis show abnormalities in approximately 70%, with ventral looping and dextrocardia as the predominant phenotypes as demonstrated by gross morphology, histology and in situ hybridization. In order to investigate the role of *Zic3* in the left-right axis pathway and the development of heterotaxy, we have intercrossed *Zic3* and *nodal* deleted heterozygous mice. *Zic3/nodal* double heterozygotes are born in significantly reduced numbers compared to heterozygous littermates. Embryonic lethality in the double heterozygotes occurs between 10.5 and 14.5 days post coitum and is associated with defects of cardiac looping which mimic those seen in the *Zic3* null mice. In contrast to *Zic3* null mice, *Zic3/nodal* double heterozygotes do not show an increased frequency of gastrulation defects. These results demonstrate that the development of heart defects in X-linked heterotaxy is a result of abnormal looping, and suggests that *nodal* signaling plays a critical role. The data further suggest that *Zic3* functions at multiple steps in the generation of left-right asymmetry, acting upstream of *T-brachyury* in the primitive streak and upstream of *nodal* in the lateral plate mesoderm.

Microarray Expression Analysis of Hepatic Transcripts of an IUGR Rat Model that Mimicks Human Syndrome
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Uteroplacental insufficiency is a common complication of pregnancy that causes low-ponderal-index intrauterine growth retardation (IUGR). IUGR is the basis for Barkers Fetal Origins of Adult Disease Hypothesis and predisposes the individual towards adult onset morbidities such as diabetes and dyslipidemia. Similar to humans, rats rendered IUGR via uteroplacental insufficiency develop insulin resistance and hypertriglyceridemia; moreover, the IUGR rat is characterized by persistent changes in hepatic gene expression and DNA methylation despite the transient nature of the initial insult. Therefore, the purpose of this study was to characterize patterns of gene expression in male IUGR rat liver versus control rat liver at day 21 of life. This project focused upon male rats at this age because they are more severely affected than female rats, but are not yet characterized by overt diabetes or dyslipidemia. Bilateral uterine artery ligation (IUGR) and sham surgery (Control) were performed to induce a well-characterized model of IUGR; litters were culled to six at birth (n=3 litters). RNA from day 21 IUGR and Control livers were used for global expression analysis using Affymetrix rat cDNA microarrays consisting of 8,784 transcripts. Uteroplacental insufficiency and subsequent IUGR increased or decreased transcript levels a minimum of two-fold in 133 and 128 genes respectively. Of particular interest, mRNA levels of enzymes involved in cholesterol biosynthesis were altered, including HMG-CoA reductase, squaline epoxidase, and 7-deoxycholesterol reductase. The promoters of these enzymes are characterized by Sp1 sites and GC richness which affect DNA methylation. Further analysis of this data identified a subset of important metabolic genes that may lend insight into the pathogenesis of in-utero programming, while suggesting possible specific targets for pharmacological intervention. This work was supported by NIH/NICHD K08HD01225 and R01HD41075.

Expression profiling in mouse neural development and differentiation. *P. Carotenuto¹, V. Aglio¹, A. Faedo², M. Cocchia¹, V. Avantaggiato¹, A. Andre¹, A. Ballabio¹, J. Rubenstein³, A. Bulfone², M. Zollo¹.* 1) TIGEM, TIGEM, Naples, Naples, ITALY; 2) SCRI, Dibit-HSR, Milan, ITALY; 3) CFNP, UCSF, San Francisco, USA.

The aim of the project is the identification and characterization of new genes specifically involved in the developing mouse telencephalon and potentially associated to human neurological disorders. Our approach combines gene array expression technology and murine subtractive cDNA library to isolate unique and specific genes preferentially expressed in the embryonic telencephalon. We have randomly sequenced 3168 cDNA clones (ESTs) from a cDNA subtractive library. Detailed sequence analysis of the selected cDNA clones by using public domain DataBases was performed, to verify the bonafide of the library, to identify human gene homologs, map and correlate them to neurological disorders; in particular, to date 28% have no public database match, and 19% correspond to genes with unknown function. A set of unique transcripts (1026) have been identified, selected and arrayed on glass coated slides. A series of experiments based on the potential of cell lines (P19, neuro2A, PC12) to be induced to differentiation into specific neuronal cell subtypes by Retinoic Acid (RA) or Neural Growth factor (NGF), have been performed. This strategy permitted us to isolate 110 genes that are differentially expressed before and after neuronal "in vitro" differentiation. To determine the spatio-temporal expression profile of 110 cDNAs, we have performed in-situ mRNA hybridization on mouse embryos (sagittal and coronal sections of E14.5 embryos and whole-mount E10.5 embryos) and adult brains. Moreover, experiments are undergoing for testing the value of this cDNA array in order to unravel the molecular defects of the developing brain of migration defect mice models.

Expression analysis supports the *CECRI* gene as a candidate for cat eye syndrome. S.A. Maier, H.E. McDermid.
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Cat eye syndrome (CES) is a human genetic abnormality characterized by defects of the eyes, anus, heart, kidney and face, as well as mental retardation. It is associated with the duplication of a 2Mb segment of 22q11.2 called the CES critical region, which is thought to contain dose-sensitive genes responsible for the CES phenotype. One promising candidate gene is *CECRI*, which is a member of a growing family of novel growth factors. Homologues of human *CECRI* have been found in various organisms, including insects, pig, zebrafish, pufferfish and baboon, but not in mouse.

In order to further characterize the *CECRI* gene, an animal model was used to determine the spatial and temporal expression pattern of *CECRI* homologues during embryonic development. Since there is no mouse homologue of *CECRI*, the pig (*Sus scrofa*) was used as a model system. Northern analysis showed *Pig CECRI* expression in adult lung and spleen. This narrow expression pattern is being complimented by using the more sensitive method of RT-PCR. *In situ* hybridization of pig embryos ranging from 22 to 31 days (equivalent to approximate mouse ages 10.5 to 13.5 days) was also used to study expression. Two different *Pig CECRI* antisense RNA probes revealed low level staining in many tissues, with significantly darker staining in the kidney, heart, liver, back, and tubules of the gut. A sense control probe from the middle of the gene was negative for all stages tested. A second sense control probe from the 3' end of the gene showed striking expression in the liver only, indicating that antisense regulation may exist. The antisense expression pattern that was found in the pig model, primarily heart and kidney, is consistent with the tissues affected in human CES, suggesting that *CECRI* may play a major role in the development of symptoms involved in CES.

Repositioning of certain muscle-specific genes to the periphery of SC35 domains during development. C.V.

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It has long been suggested that gene organization within the nucleus may facilitate the coordinate regulation of thousands of genes during development and differentiation of specific cell-types. Previous studies have shown that in a given cell-type certain active genes associate with SC35 domains, nuclear regions rich in RNA metabolic factors and excluded from heterochromatin. This organization is locus-specific and not a property of all active genes. For example, the myosin heavy chain gene is essentially always associated with SC35 domains in muscle cells, whereas the dystrophin gene/RNA are not. To better understand and establish the relationship of this organization to gene expression, it is important to investigate the distribution of the same genes in different cell-types during development. Here we investigate whether gene organization relative to SC35 domains is cell-type specific by following several muscle and non-muscle genes in fibroblasts, committed but proliferative myoblasts, and terminally differentiated muscle. While no change was seen for other loci, two muscle genes (human b-cardiac myosin heavy chain and myogenin) repositioned to the immediate periphery of an SC35 domain in differentiated muscle nuclei. Similar results were observed for mouse 3T3 cells induced to form muscle by introduction of MyoD. For those genes that changed position relative to SC35 domains, brief analysis of gene position relative to the chromosome territory or centric heterochromatin showed no repositioning relative to these structures. Results demonstrate a cell-type specific reorganization of specific developmentally regulated loci relative to discrete nuclear domains of RNA metabolic factors.

Mutation spectrum in the Opitz syndrome gene (MID1), its expression during human development and X inactivation pattern in severely affected females . L. PINSON, J. AUGÉ, S. AUDOLLENT, G. MATTEI, N. GIGAREL, A. MUNNICH, S. LYONNET, M. VEKEMANS, T. ATTIE-BITACH. Departments of Genetics, INSERM U393, Hospital Necker, Paris, FRANCE.

Opitz syndrome (OS) is a midline malformation syndrome characterized by hypertelorism, hypospadias, oral, oesolaryngotracheal and anal defects. A heart malformation is observed in 25% of patients. Recently, MID1 mutations have been identified in the X-linked form of the disease, while the gene of the autosomal dominant form on 22q11 remains unknown. Here, we report on the spectrum of MID1 mutation in our 14 OS patients series, X inactivation pattern in 3 severely affected females and the expression pattern of MID1 during human development. X inactivation pattern was studied in 3 severe OS females. A skewed pattern of inactivation (91/9%) in one girl strongly suggested X linked inheritance. SSCP analysis of the 9 exons of the MID1 gene followed by sequencing of 9 abnormal patterns detected mutations in 7/14 patients (5 familial and 2 sporadic cases). All were novel mutations except one (R495X) previously reported in one patient (Hum. Mol. Genet. 2000, 9 :2553-2562). Interestingly, both patients presented with vermis hypoplasia, a rare feature of OS. Two different MID1 changes were detected in a severely affected girl presenting a random pattern of X inactivation. Further experiments are underway to see whether the two changes are carried by the same or different alleles. Finally, in order to compare the pattern of MID1 expression with the clinical features of the disease, we performed in situ hybridization on human embryos. MID1 was mainly expressed in the central nervous system, oropharynx, oesotracheal epithelia, genital tubercle and anal membrane. MID1 expression was also observed in kidney, limb buds, but unlike the mouse, it was expressed in a restricted area of the heart interventricular septum, thus correlating with occasional features of the syndrome. This study supports the broad spectrum of MID1 mutations, the major involvement of MID1 in OS and confirms that vermis hypoplasia is a feature of the syndrome. Finally, expression studies suggest involvement of MID1 in human heart development.

EDA targets revealed by skin gene expression profiles of wild-type, Tabby, and Tabby EDA-A1 transgenic mice.

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Mutations in the EDA gene cause Anhidrotic Ectodermal Dysplasia (EDA), with lesions in skin appendage formation. To begin to analyze EDA pathways, we have used expression profiling on 15,000-gene mouse cDNA microarrays, comparing adult mouse skin from wild-type, EDA-defective (Tabby), and Tabby mice supplemented with the EDA-A1 isoform, which is sufficient to rescue multiple Tabby phenotypes. Given the sensitivity of the current microarray system, 8,500 genes (60%) were estimated to be expressed, including transcription factors and growth-regulatory genes that had not previously been identified in skin; but only 24 (0.16%), one third of them novel, showed significant differences between wild type and Tabby. An additional 8 genes not included in the 15K gene set were shown to have expression differences by real time RT-PCR. Sixteen of 32 affected genes were restored significantly toward wild-type levels in EDA-A1 transgenic Tabby mice. Significant up-regulation in Tabby skin was observed for several dermal matrix genes, including *Col1a1*, *Col1a2*, *Col3a1* and *Sparc*. In contrast, down-regulation occurred for the NEMO/NF- κ B pathway, already implicated in skin appendage formation, and even more markedly for a second pathway, JNK/c-jun/c-fos and their target genes, that has not previously been clearly associated with skin development. These data are consistent with the regulation of the NF- κ B pathway by EDA, and support its involvement in the regulation of the JNK pathway as well.

The characterization of genes with different expression levels in mouse blastocyst inner cell mass cells and trophoctoderm cells. *J.C.F.M. Dreesen¹, M. Bras², J.G. Derhaag², J.C.M. Dumoulin², E. Coonen², H.J.M. Smeets¹, J.L.H. Evers², J.P.M. Geraedts¹, J. Herbergs¹.* 1) Clinical Genetics, Academic Hospital Maastricht, Maastricht, The Netherlands; 2) Obstetrics and Gynaecology, Academic Hospital Maastricht, The Netherlands.

The blastocyst represents the embryonic stage in which differentiation of embryonic cells into trophoctoderm (TE) and the inner cell mass (ICM) has occurred. Using immunosurgery and mechanical separation, ICM- or TE-cells can be divided and isolated from the blastocyst. In this study blastocysts were obtained from superovulated (C57BI/6XCBA)F1 mice in vivo fertilized by random-bred Swiss males. ICM- and TE-cells were isolated from single blastocysts. mRNA was purified and cDNA was transcribed and amplified with the SMART cDNA synthesis kit. Differential display PCR was performed on replicate ICM, TE samples and total blastocyst cDNA samples, followed by separation of the PCR fragments on a denaturing polyacrylamide gel. ICM- and TE-specific bands as well as shared bands present in ICM, TE and total blastocysts (controls) were isolated from the gel, amplified and sequenced. Sequences were annotated by BLAST searches in the commercial CELERA and public NCBI and MGD nucleotide sequence databases. 25 of the 52 isolated fragments could be directly sequenced and annotated according to NCBI nomenclature. Comparison of TE-cells with ICM-cells showed differential expression of five of 7 identified genes and 2 of 3 anonymous cDNA sequences. Furthermore, three control sequences equally expressed in the blastocyst, ICM- and TE-cells, showed consistent results with respect to sequencing and annotation. In conclusion we have isolated, sequenced and annotated sequences from transcripts differentially expressed in ICM- and TE-cells. Equal expression of control sequences showed the feasibility of this method. Differentially expressed genes are currently being confirmed and will be quantified by quantitative PCR analysis and evaluated for their role in blastocyst formation and implantation.

Isolating New Genes of Adrenal Development. *S. Ching*¹, *P. Dewing*¹, *E.R.B. McCabe*^{1, 2}, *E. Vilain*^{1, 2}. 1) Human Genetics, UCLA, Los Angeles, CA; 2) Pediatrics, UCLA, 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 was 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 15.5 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. Two of these genes, serum amyloid P-component (SAP) and L-FABP (liver fatty-acid binding protein), showed a 10.3-fold and 13.4-fold increase, respectively. RT-PCR confirmed that there were highly significant differences in mRNA transcript levels between 15.5 dpc, P0 and P8, with a strong peak occurring around P0. SAP is a member of the pentraxin family of proteins which binds to apoptotic cells and interacts with membrane receptors on phagocytes to mediate phagocytosis. L-FABP belongs to the family of hydrophobic carrier proteins and has been implicated as a regulator of mitogenesis in rat hepatocytes. These results suggest that SAP and L-FABP are involved in remodeling the developing adrenal glands, and therefore in the pathogenesis of AHC.

Vsx1, a rapidly evolving paired-like homeobox gene is essential for retinal cone bipolar cell development. *R.R. McInnes¹, R.L. Chow¹, D.G. Birch²*. 1) Program in Developmental Biology, Research Institute, Hospital Sick Children, Toronto, ON, Canada; 2) Retina Foundation of the Southwest, Dallas, Texas.

VSX1 and CHX10 are closely related paired-like homeodomain transcription factors expressed in the vertebrate retina. Consistent with the retinal expression of Chx10 during both early and late stages of eye development, Chx10^{-/-} mice (and humans) have profound developmental eye defects: microphthalmia, blindness, poor proliferation of retinal progenitors, and an absence of both rod and cone bipolar cells, the major class of retinal interneurons. In contrast, Vsx1 is expressed only at late stages of eye formation, and solely in "OFF" and "ON" cone bipolar cells. Despite the absence of Vsx1 expression in adult mouse & human cornea, human VSX1 missense mutations are associated with the dominant corneal disorders posterior polymorphous dystrophy & keratoconus. To determine the role of Vsx1 in eye development and bipolar cell biology, and in the pathogenesis of the monogenic corneal disorders, we generated Vsx1^{-/-} mice with a tau-lacZ reporter gene knock-in. In Vsx1^{-/-} mice, cone bipolar cells were normally specified, since tau-lacZ expression was detected in a pattern characteristic of both "OFF" and "ON" cone bipolars. In the Vsx1^{-/-} retina, however, normal differentiation of cone bipolar cells was disrupted, since expression of the cone bipolar markers recoverin and caldendrin was lost, and the axonal termini of these cells were morphologically abnormal. Unexpectedly, no corneal expression was detected during mouse development and no adult corneal abnormalities were noted. We conclude that 1) Vsx1, unlike Chx10, is not required for early eye development, 2) Vsx1 is essential for the late differentiation program of retinal cone bipolar cells in mammals, 3) Vsx1^{-/-} mice and humans are likely to have serious vision defects due to the disruption in cone bipolar formation, 4) The role of Vsx1 in the cornea remains unclear, but the Vsx1^{-/-} mouse mutant will serve as a valuable model system into which the human VSX1 mutations can be introduced, to begin to explore the pathogenesis of the human corneal defects.

Tissue specific expression of type X collagen in hypertrophic chondrocytes is specified by *cis* elements in both the *Col10a1* promoter and second intron. Q. Zheng¹, G. Zhou¹, Y. Chen¹, A. Parker², B. Lee¹. 1) Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Respiratory and Inflammation Research Area, AstraZenica, Cheshire, United Kindom.

Type X collagen is a specific marker for hypertrophic chondrocytes and is altered in Schmid metaphyseal chondrodysplasia. Until recently, no transcriptional determinants specifying chondrocyte-specific type X collagen gene (*Col10a1*) expression have been identified. We have characterized the 4 kb murine *Col10a1* promoter region and demonstrated that it contains conserved *Runx2/Cbfa1* binding sites. We show that these *cis* elements bind RUNX2, an essential RUNT domain transcription factor required for osteoblast differentiation and chondrocyte maturation, and can mediate transactivation of the *Col10a1* minimal promoter both *in vitro* and *in vivo* in reporter transgenic mice. However, further analysis of our transgenic mice showed that the 4 kb element is required but not sufficient for high level tissue-specific expression, since the reporter was primarily localized in the lower hypertrophic zone. We hypothesized that additional positive and/or negative *cis* elements outside of this 4 kb promoter are required for high level specific expression throughout the zone of hypertrophy. We report that an 8 kb *Col10a1* region encompassing the same proximal promoter and large second intron can direct reporter gene (*LacZ*) expression throughout the zone of hypertrophy. A stable MCT cell line containing this *Col10a1* 8 kb element upstream of a reporter shows 7 fold up-regulation of reporter activity when cells undergo hypertrophy. Interspecies sequence analysis of *Col10a1* identified a highly conserved element that may serve as a tissue-specific enhancer within *Col10a1*. Electrophoretic mobility shift assay (EMSA) using nuclear extracts from hypertrophic MCT cells confirmed formation of a specific DNA-protein complex. The identification of additional transacting factors that regulate *Col10a1* will lead to a better understanding of skeletal dysplasias that affect metaphyseal long bone development.

Paternally transmitted recurrent tetragametic chimerism resulting in recurrent true-hermaphroditism. C.

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This report describes a family displaying paternally transmitted predisposition to tetragametic chimerism. The family has 4 individuals affected with 46,XX true hermaphroditism spanning two generations and displays a paternal inheritance pattern. Previous Southern analysis of lymphocyte DNA ruled out the presence of a Y- to X- or autosomal translocation or of a Y-chromosomal inversion that could lead to recurrent translocations. Pathological examination of excised gonadal tissue from affected individuals indicated that they were in fact true hermaphrodites with ovotestes. Subsequent immunohistochemical studies of the paraffin-embedded gonadal tissue revealed a mosaic pattern of SRY gene expression. Here, we report short tandem repeat analyses of the blood and gonadal tissue of two affected individuals and their parents. Normal bi-parental inheritance of X-chromosomal and autosomal markers was observed in lymphocyte DNA from both individuals. Multiple Y-chromosomal markers were present in SRY-expressing gonadal tissues suggesting the presence of an intact Y-chromosome. Bi-parental inheritance of X-chromosomal markers was also observed in gonadal tissue; however, in some regions of the tissue, an additional maternal allele was observed. Most commonly the autosomal alleles observed in the ovotestes were the bi-parental alleles observed in lymphocyte DNA. In some instances, additional autosomal alleles of maternal or paternal origin, or both, were seen in the ovotestes. Occasionally, no autosomal alleles from one parent were present in the ovotestes. Taken together these data suggest that the paternally transmitted phenotype in this family is predisposition to formation of tetragametic chimeras. Tetragametic chimeras arising from sperm with different sex chromosomes develop as true hermaphrodites with ambiguous genitalia that brings them to clinical recognition. The observation that all alleles from one or the other parent may be lost in some parts of the tissue suggests that there is chromosomal instability in some cells.

Analysis of DNA-Binding Specificity of the Forkhead Transcription Factor FOXC1, a Gene Mutated in Axenfeld-Rieger Malformations. *T.C. Murphy, R.A. Saleem, M.A. Walter.* Ophthalmology/Medical Genetics, University of Alberta, Edmonton, Alberta, Canada.

FOXC1 is a developmentally important transcription factor. Mutations in FOXC1 lead to aberrant development of the anterior segment of the eye and increase the risk of early-onset glaucoma. In order to better understand the mechanisms of FOXC1 function and its role in ocular development, we have undertaken an analysis of the DNA binding specificity of FOXC1. FOXC1 has been shown to interact with an *in vitro* derived DNA binding site (GTAAATAAA), while the distantly related FOXH1 binds the *in vitro* preferred site TGTG/TG/TATT. We are using these differences in DNA-binding specificities in domain swapping experiments to define the amino acids of the forkhead domain that regulate the DNA binding specificity of FOXC1. The 21 amino acids immediately upstream of helix 3 in forkhead proteins have been suggested to be involved in the regulation of DNA binding specificity. We are using site-directed mutagenesis to convert these 21 amino acids within the FOXC1 cDNA to reciprocal amino acid positions from the forkhead domain of FOXH1. These 21 amino acids will be swapped stepwise in a series of three constructs each comprised of 7 amino acids from FOXH1. The effect of the FOXH1 amino acids within the context of FOXC1 forkhead domain will be tested by evaluating the relative affinities of the chimeric proteins for the FOXC1 and FOXH1 binding sites by electrophoretic mobility shift assays (EMSAs) and transactivation of luciferase reporter construct containing either the FOXC1 or FOXH1 binding sites. Preliminary results indicate that a FOXC1-H1 chimeric protein in which the anterior most 7 amino acids preceding helix three have been converted from FOXC1 to FOXH1 displays altered site specificity on a panel of oligonucleotides that are variants the FOXC1 binding site. Analyses of the remaining FOXC1-H1 chimeric proteins are in progress. This work will help to precisely delineate the specific residues involved in DNA site selection. A mechanistic understanding of how FOXC1 recognises and binds to DNA will be critical to defining the function of FOXC1 in the development of the eye.

Localization of the mouse *Tcm* gene to a 1Mb region on chromosome 4. *K.S. Wang*^{1,2}, *P. Grimes*², *A. Dutra*³, *J. Favor*⁴, *D. Stambolian*^{1,2}. 1) Department of Genetics, University of Pennsylvania, Philadelphia, PA; 2) Department of Ophthalmology, University of Pennsylvania, Philadelphia, PA; 3) National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 4) GSF-Neuherberg, Institute of Mammalian Genetics, National Research Center for Environment and Health, Neuherberg, Germany.

Tcm (total cataract with microphthalmia) is an autosomal dominant mouse mutation recovered from an X-irradiated mouse. Initial characterization of the heterozygous *Tcm* mouse revealed total lens opacity, microphthalmia, iris dysplasia, and ventral coloboma. We have recently characterized the homozygous *Tcm* phenotype which results from a failure of ventral retinal development, leading to retinal dysplasia and subsequent anophthalmia. Variable brain abnormalities include hydrocephalus, cerebellar white matter cysts, and possible absence of the septum pellucidum. Spectral karyotyping analysis was done on *Tcm* mouse chromosomes and found to be normal. In this study, we report on the use of a mouse backcross breeding strategy (C3Hx102 x C57BL/6) with analysis of over 270 resultant progeny using microsatellite markers D4Mit101, D4Mit106, D4Mit235, D4Mit263, and D4Mit264 to localize *Tcm* to an approximately one megabase region on mouse chromosome 4. This locus is delineated by microsatellite markers D4Mit235 and D4Mit264 with *Tcm* mapping approximately 0.7 cM and 0.4 cM from each marker, respectively. We also report on the evaluation and exclusion of several candidate genes including *Mmp16*, *Cbfa2t1h*, and *Gem* by Southern and Northern analysis. Additional developmental characterization of the mutation is also presented to further define the timeframe in which the *Tcm* phenotype manifests.

Abnormal diaphragmatic development and pulmonary hypoplasia caused by an ENU-induced hypomorphic mutation of *Fog2*. K.G. Ackerman¹, B.J. Herron¹, C. Rao¹, H. Huang¹, R.P. Babiuk², J.A. Epstein³, J. Greer², D.R. Beier¹. 1) Genetics Div., Brigham and Women's Hospital, Boston, MA; 2) Div. of Neuroscience, U. of Alberta, Edmonton, AB; 3) Cardiovascular Div., U. of Pennsylvania Medical School, Philadelphia, PA.

We are screening progeny of ENU-mutagenized mice to identify recessive mutations causing developmental abnormalities similar to those seen in human congenital defects. In an examination of day 18.5 embryos we identified a mutation that results in severe pulmonary hypoplasia and abnormal diaphragmatic development. Specifically, while the diaphragm is intact, its dorsolateral portion fails to muscularize. Genetic mapping was used to localize the mutation to a 1.1 cM interval on chromosome 15. This localization facilitated the identification and examination of homozygous mice at earlier timepoints during embryonic development, which revealed that approximately half the mice homozygous for the mutation die *in utero* between day 12.5 and day 15.5. The affected mice have cardiac defects identical to those previously reported for a targeted mutation of Friend of Gata 2 (*Fog2*), which is in the recombinant interval. Molecular analysis identified a point mutation in a splice donor site that results in an insertion of 85bp of intronic sequences into the *Fog2* transcript, resulting in early termination and a severely truncated protein product. The causal role of the ENU-induced mutation was proven by demonstrating that it is non-complementing in a cross with a *Fog2*-null mutant mouse.

Our results demonstrate the utility of a phenotype-driven strategy for understanding the genetic contributions to mammalian development. The importance of *Fog2* for normal cardiac development has been previously shown, but its role in diaphragm and lung development was not recognized. Additionally, the fact that a defect in *Fog2* affects diaphragm, lung, and heart development may be relevant to the syndrome of congenital diaphragmatic defect (CDD) in humans, as CDD has been reported in 2 unrelated patients with balanced translocation breakpoints at 8q22.3, the location of human FOG2.

Expression and functional analyses of zebrafish *tgfb3*. *F.S.H. Cheah*^{1,5}, *E.W. Jabs*^{3,4,5}, *S.S. Chong*^{1,2,3,5}. 1) Pediatrics; 2) Obstetrics & Gynecology, and Laboratory Medicine / Molecular Diagnosis Center, National University of Singapore and Hospital, Singapore; 3) Pediatrics, Center for Craniofacial Development and Disorders, Institute of Genetic Medicine; 4) Medicine, and Plastic Surgery, Johns Hopkins University School of Medicine, Baltimore, MD; 5) Johns Hopkins Singapore, Singapore.

Transforming growth factors (TGFs) constitute one of the many extracellular signaling molecules that display a remarkable spectrum of effects on patterning, epithelial-mesenchymal interactions, cell proliferation, apoptosis, and chondrogenesis in both vertebrates and invertebrates. In particular, TGF β 3 is known to contribute to facial development, especially in palate formation. The nucleotide sequence of the full-length zebrafish *tgfb3* cDNA has not been determined, and its normal expression pattern during zebrafish development has not yet been characterized. Here we report the complete sequence of the zebrafish *tgfb3* cDNA and its embryonic expression pattern. In addition, *tgfb3* morphants were generated by antisense morpholino targeting technology. Day 4 post-fertilization morphants were generally shorter than their normal AB counterparts. Their ear vesicles were also smaller and some had an enlarged pericardial space. Alcian blue staining revealed malformation of both the head and pharyngeal arches. All neurocranial components, including the ethmoid plate and trabeculae, were severely reduced. Parachordal and auditory capsules were also absent. The first pharyngeal arch pair was severely reduced and malformed, the second to sixth pairs were severely shortened, while the seventh pair was completely absent. Furthermore, the basibranchial and hypobranchial elements were also absent. These data suggest that *tgfb3* may be involved in zebrafish craniofacial development.

Phenotypic characterization and positional cloning of craniofacial mutants generated from a recessive ENU mutagenesis screen. *B.C. Bjork*¹, *B.J. Herron*¹, *M.J. Justice*², *J.D. McDonald*³, *D.R. Beier*¹. 1) Division of Genetics, Brigham & Women's Hospital, Harvard Medical School, Boston, MA; 2) Baylor College of Medicine, Houston, TX; 3) Wichita State University, Wichita, KS.

ENU mutagenesis has proven to be a powerful means by which to generate single nucleotide mutations in mice. We are screening E18.5 embryos from ENU-treated mice for recessive phenotypes that resemble human congenital disorders. The screen is performed as an outcross to facilitate the rapid localization of mutant loci using a strategy of interval haplotype analysis. Among the large spectrum of phenotypic abnormalities we have identified are defects involving craniofacial development, including nonsyndromic and syndromic cleft primary and secondary palate.

Orofacial clefting is a major human congenital disorder with a complex etiology and a high incidence (1/500 to 1/2500 live births). Many known mouse mutants contain orofacial clefts as part of their phenotype, but the best models for human clefting are those with clefts in the absence of additional phenotypes. In an analysis of 54 families for recessive mutations, we identified four models of orofacial clefting including *cleft palate only 1* (*cpo1*; cleft secondary palate with no additional phenotypic abnormalities), *curly tail 2* (*ct2*; variable expression of cleft secondary palate, a curly or kinked tail and bent forelimbs) and *little chin* (cleft secondary palate and a small mandible that resembles the abnormalities observed in the Pierre Robin syndrome). Most recently, we identified *cleft lip/palate 1* (cleft primary and/or secondary palate with no additional phenotypic abnormalities). Critical regions of 500 kb on distal chromosome 4 and 8 cM on proximal chromosome 3 are defined for the *cpo1* and *ct2* mutations, respectively. Using novel polymorphic markers and the public mouse genomic sequence, we continue to refine these genetic intervals and to identify and prioritize candidate genes for mutation screening. We report the genetic localization, fine-mapping, developmental characterization and candidate gene screening for these ENU-induced recessive mutations.

Developmental mechanisms of congenital vertebral malformations in the *Dll3-pudgy* mouse mutant homolog of *DLL3*-spondylocostal dysostosis. K. Kusumi^{1,2}, M.S. Mimoto¹, K.L. Covello², S.A. Stevens¹, S.L. Dunwoodie³. 1) Division of Human Genetics & Molecular Biology, The Children's Hospital of Philadelphia, Phila., PA; 2) University of Pennsylvania School of Medicine, Philadelphia, PA; 3) Victor Chang Cardiac Research Institute, Darlinghurst, NSW 2010 & Department of Biotechnology and Biomolecular Sciences, University of New South Wales, Australia.

The notch ligand, delta-like 3 (*Dll3*), is disrupted in the mouse pudgy (*Dll3^{pud}*) and targeted (*Dll3^{neo}*) mutants and in the human vertebral segmentation disorder, spondylocostal dysostosis (SD, MIM 277300). Disruptions of delta-like 3 in both human and mouse are characterized by generalized axial vertebral and rib malformations. To identify the developmental origins of these defects, we have examined somitogenesis, the embryological process generating axial segments, in *Dll3^{pud}* mutants. *Dll3^{pud}* mutant embryos have defective epithelial somite segmentation and fail to form normal rostral-caudal somite domains, leading to vertebral malformations. Initiation of somite formation and *Lfng* dynamic expression is observed in mutants, but there is a failure to maintain cycling. The somite cycling genes *Hes1*, *Hes7*, and *Hey3* were also examined in mutant embryos, and major disruptions were observed both at the onset of segmentation and in mid-somitogenesis. These *Dll3^{pud}* results are consistent with observations of somitogenesis in the *Dll3^{neo}* null allele. In summary, the *Dll3* gene appears to be required for maintenance of normal cycling in somitogenesis and initial expression of some notch pathway genes. Given segmental defects observed in targeted mutations in many notch pathway genes, defects in these loci may be involved in the genetic etiology of human congenital vertebral malformations, including congenital scoliosis, congenital kyphosis, Klippel-Feil syndrome, and Jarcho-Levin syndrome, and VATER and VACTERL associations.

Influence of the ion channel polycystin-2 on organ morphogenesis and left-right axis development in mice. J.

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The establishment of left-right asymmetry can be divided into initial breakage of symmetry, establishment of asymmetrical gene expression and transfer of this positional information to the developing organs. Embryological and genetic experiments revealed a conserved asymmetric signaling cascade, which during early embryogenesis transmits asymmetric cues from the embryonic midline to the lateral plate mesoderm and the forming organs. The central players in this scheme are four asymmetrically expressed genes: nodal, Lefty1 and Lefty2, and Pitx2c. Recently we have generated a Pkd2 knockout mouse. Pkd2 encodes polycystin-2, an intracellular Ca-release channel expressed in the ER membrane. In humans mutations in PKD2 account for 15 % of autosomal dominant polycystic kidney disease (ADPKD) which is one of the most frequently inherited monogenic diseases and affects up to 1:1000 individuals worldwide. ADPKD is characterized by progressive development of fluid filled cysts in the kidneys that frequently result in end-stage renal failure. Loss of Pkd2 causes recessive embryonic lethality. Pkd2^{-/-}LacZ^{+/+}-embryos develop whole body oedema and cysts in the kidneys, pancreas and liver and exhibit skeletal malformations. All mutant hearts analyzed so far exhibited structural deformations with abnormalities in trabecularisation and cardiac septation. Surprisingly, Pkd2 knockout mouse embryos displayed left-right positional defects: embryonic turning, heart looping and placement of abdominal organs were randomized and the lung showed right pulmonary isomerism. Lefty1, Lefty2 and nodal were not expressed in the left lateral plate mesoderm and Pitx2 was absent from heart and lung primordia and bilaterally expressed in body wall, mid and hindgut. The embryonic midline was present and normal levels of hnf3 and shh were expressed. We suggest that polycystin-2 acts upstream of the nodal cascade in left-right axis determination and that Pkd2 might be one of the master genes of the body plan.

Altered Expression of Late-Gestation-Lung 1 (LGL1) in a Rat Model of Bronchopulmonary Dysplasia (BPD). *K. Nadeau*^{1,2,3}, *R. Jankov*^{4,5,6}, *K.A. Tanswell*^{4,5,6}, *N.B. Swezey*^{5,6,7}, *F. Kaplan*^{1,2,3,8,9}. 1) Dept of Human Genetics; 2) McGill University, Montreal, QC, Can; 3) Mont. Child. Hosp. Res. Inst, Montreal, QC, Can; 4) Lung Biology and Medical Research Council Group in Lung Development; 5) Hospital for Sick Children Res. Inst., Toronto, ON, Can; 6) University of Toronto, ON, Can; 7) Dept. of Pediatrics and Physiology; 8) Dept. of Pediatrics; 9) Depts. of Pathology and Pediatrics, Harvard Med School, Boston, MA, USA.

Deficiency in terminal lung differentiation due to premature birth is a leading cause of morbidity in the newborn period (RDS), with sequelae that can extend into adulthood (BPD). Although multiple pathophysiologic mechanisms are likely to contribute to BPD, it is believed to be largely due to an arrest in alveolar development. LGL1 is a glucocorticoid induced and developmentally regulated gene cloned in our laboratory. It is maximally expressed in rat lung mesenchymal cells at fetal day 21, concordant with the onset of alveolar septation and augmented surfactant production. We therefore hypothesized that arrested alveolarization in BPD may be associated with altered expression of LGL1. To test this hypothesis, we investigated alterations in LGL1 RNA (northern analysis, n=2) and protein (immuno-histochemistry, n=1) in a rat model of BPD generated by exposing neonatal rats to 60% oxygen for 7, 10 or 14 days (n=4 litters/group). Control litters were exposed to air for the same time periods (n=4 per group). We observed decreased levels of LGL1 mRNA in the BPD rats compared to controls at each time point. A diminution in Ig11 protein level is apparent in control lungs between fetal days 7 and 14. These data confirm our original finding that Ig11 expression diminishes with time after its peak expression at fetal day 21. We showed by immunohistochemistry that Ig11 protein is further reduced in the BPD rats compared to controls at neonatal days 7 and day 14. We have previously shown that absence of Ig11 in the pseudoglandular lung affects lung branching. Our current findings support an additional role for LGL1 during the period of alveolarization. We speculate that deficiency of Ig11 contributes to the arrested alveolar partitioning observed in BPD.

Using cDNA Microarray Analysis to Understand Pathways Related to Waardenburg Syndrome. *S.R. Lipner¹, L. Kochilas², J.A. Epstein², B.E. Morrow¹, A. Skoultchi¹*. 1) Albert Einstein College of Medicine, Bronx, NY; 2) University of Pennsylvania Medical School, Philadelphia, PA.

Waardenburg syndrome (WS) is an autosomal dominant disorder with an incidence of 1 in 40,000 live births. Patients with WS have sensorineural deafness, pigmentation anomalies, cardiac and skeletal abnormalities and neural tube defects. WS1 and 2 are caused by loss of function mutations in Pax3. Mutations of Pax3 have been identified in splotch (sp) mouse strains and these mice have characteristics similar to WS patients. Heterozygotes have a white belly spot and homozygotes die at E13.5 with spina bifida, craniofacial abnormalities, conotruncal heart defects, and thymus and thyroid abnormalities. Many of the organs and tissues that are affected in WS patients and splotch mice are derived from neural crest cells. Another mouse strain that has defects in tissues derived from the neural crest is the Hoxa3 knockout mouse. Many of the defects seen in Hoxa3 homozygotes resemble those seen in Pax3 homozygotes. Hoxa3 homozygotes die at birth with craniofacial malformations, as well as cardiovascular, thymus, thyroid and parathyroid defects. We are interested in understanding the relationship between Pax3 and Hoxa3, including the downstream pathways in which their effects may intersect. Our approach is to use semiquantitative RT-PCR and cDNA microarrays to identify genes whose expression is changed in Pax3 and Hoxa3 mutant embryos. Our data indicate that Pax3 and Hoxa3 do not lie either upstream or downstream of each other. However, Hoxa3 and Pax3 may be in parallel pathways and regulate some of the same genes. One of these genes is Trp-2, which is expressed in migrating melanoblasts of mouse embryos beginning at E10.5. We believe that Trp-2 is activated by both Hoxa3 and Pax3, since this gene is downregulated in both types of knockout embryos. In addition, we have shown that Hoxa3 and Pax3 are coexpressed with Trp-2 in B16 melanoma cells. We are currently studying the mechanism by which these transcription factors regulate Trp-2 gene expression.

Gene expression profiles of human craniofacial development. *Y. Korshunova*¹, *R. Tidwell*¹, *R. Veile*¹, *D. Messina*¹, *C. Helms*¹, *M. Lovett*¹, *T. Attié-Bitach*², *J. Augé*², *S. Audollent*², *M. Vekemans*², *D. Ash*³, *J. Cai*³, *E.W. Jabs*³. 1) Washington Univ, St Louis, MO; 2) Hospital Necker Enfants Malades, Paris; 3) The Johns Hopkins Univ, Baltimore, MD.

Our consortium is profiling gene expression changes in human craniofacial development to identify new lineage markers, to identify important pathways and to pinpoint genes that might contribute to craniofacial disorders. We are using multiple methods to generate profiles from 25 microdissected structures spanning 4 weeks through 8.5 weeks of development. This collection of tissues includes many defined lineages. The example below illustrates data derived for the frontonasal prominence (FNP) lineage which eventually gives rise to the nose and parts of the upper lip. By analyzing multiple hybridizations to Affymetrix chips we identified 173 genes that are discretely expressed in four FNP structures. Sixty four genes, including the homeobox D9, the zinc finger 197, basic transcription factor 3 and homeobox D1 genes are specifically upregulated in the 4th week FNP. Twenty six genes are specific to the 5th week FNP including thrombospondin 2 and catenin delta 1. Thirty six genes are upregulated in the 6th week medial nasal prominence including thrombospondin 4 and zinc finger 267. Forty five genes are specific to the 6th week lateral nasal prominence. These include zinc finger 208 and basic transcription factor 2. We have also interrogated our own custom built microarray of 50mer oligonucleotides. This 1700 gene array corresponds to all known (and several previously unknown) human transcription factors. All pair-wise comparisons for the tissues in our collection were performed and revealed additional transcription factors that show differential expression profiles. For the FNP lineage, TBX21, BARX1, BAPX1, EMX2, SOX10, MNT, ID1 and BRPF1 show distinct temporal changes. Many of these changes have been validated by RNA in situ hybridizations to embryo sections and to whole mount mouse embryos. In the longer term, analysis of this dataset should prove useful as one route for identifying pathways and genes that contribute to common craniofacial defects such as cleft lip and palate.

***Cops3* is essential for murine embryonic development.** J. Yan¹, K. Walz¹, S. Carattini-Rivera¹, A. Bradley², J.R. Lupski¹. 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Sanger center, Cambridge, UK.

Cops3 is located in the common deletion interval for the Smith-Magenis Syndrome (SMS), a contiguous gene deletion syndrome. It encodes the third subunit of the COP9 signalosome, which is an eight-subunit protein complex and is similar to the regulatory lid of the 26S proteasome. The COP9 complex was originally identified as a repressor of light mediated development in plant and mutants of this complex are lethal after the seedling stage. Disruption of one of the subunits in *Drosophila* caused lethality at the late larval or pupal stages. In order to investigate its potential role in mammals and in the SMS phenotypes, we disrupted the murine *Cops3* by an insertional vector. The heterozygous mice appear normal, although the protein level is reduced. In 18 offsprings from the heterozygous mice intercrosses, no homozygous mice were observed. Embryos from heterozygous intercrosses were then examined. At 13.5 days postcoitum (dpc) and 10.5 dpc, no homozygous embryos were detected. At 9.5 dpc, morphologically abnormal embryos have been found, while at 6.5 and 7.5 dpc, all the embryos looked normal. It seems the *Cops3* homozygous embryos died between 7.5 dpc and 10.5 dpc. *Cops3* is crucial for maintaining the development of the embryo in mice. Further analysis is underway to reveal the cause of lethality.

Basic helix-loop-helix transcription factors Twist and Dermo1 during palatal, tooth, and calvarial development.

Y.L. Wang, W.A. Paznekas, L.E. Kotch, E.W. Jabs. Inst. Genet. Med., Johns Hopkins Univ., Balto., MD.

Twist and Dermo1 are closely related members of the family of basic helix-loop-helix (bHLH) transcription factors. The human TWIST (202AA) and DERMO1 (160AA) proteins have 66 percent AA identity. We sequenced these genes and found that nucleotides 127-159 and 173-483 of DERMO1 cDNA exhibit 93 and 89 percent identity to n199-231 and 299-609 of TWIST. The mouse Twist (206AA) and Dermo1 (160AA) proteins have 65 percent AA identity, and n127-158 and 168-483 of Dermo1 cDNA exhibit 90 and 87 percent identity to n199-230 and 306-621 of Twist (n1, first base of the start codon). We analyzed and compared the distribution of Twist and Dermo1 transcripts during mouse craniofacial development from E13.0-E17.0 in vivo by in situ hybridization with digoxigenin-labeled cRNA probes designed from nonhomologous regions of each gene transcript. Dermo1 and Twist exhibited similar dynamic expression patterns in the developing palate, tooth, calvarial bone and other craniofacial organs. They appeared to be expressed at sites of epithelial-mesenchymal interaction during palate and tooth development. At E13.0 both genes were expressed strongly in the mesenchymal and epithelial cells throughout the entire palate and tooth bud primordia. From E15.0 to E17.0 the expression in mesenchyme decreased and the transcripts were primarily located in palate-derived epithelial cells lining both oral and nasal cavities, as well as the tooth bud epithelium of the enamel organ. These data suggest that Dermo1 may play a complementary role in the same embryonic craniofacial processes as Twist, and given their spatial overlap in distribution they may play a role in the epithelial-mesenchymal interactions that dictate development of craniofacial organs. As both are expressed in osteoblastic cells and mutations in the TWIST gene were found in patients exhibiting the Saethre-Chotzen syndrome phenotype which includes premature fusion of the coronal sutures, we examined a 693bp region encompassing the coding sequence of DERMO1 in 33 patients classified as having Saethre-Chotzen syndrome or coronal craniosynostosis, no mutations or polymorphisms were revealed.

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Expression of CTCF gene in preimplantation embryos. *A.F.L. Rios, D.C. Lemos, M.B. Fernandes, M.V. Andrea, R.B. Lobo, M. Mazucato, E.S. Ramos.* Dept Genetics, Fac Med Rib Preto - University of Sao Paulo, Ribeirao Preto, Sao Paulo, Brazil.

CTCF, a conserved factor, is involved in X chromosome inactivation and expression of imprinted genes. It plays a central role in the epigenetics and cell growth regulations, and perturbations of its functions occur in some forms of neoplasias. Experimentation with embryos has become an important breakthrough in medical research. However, it is also a source of controversy, because the ethical problems. We have identified in cattle a sequence of the CTCF gene. Molecular analysis and characterization of the sequence document homology to its human counterpart. In the present study, the RT-PCR was employed and transcripts were detectable during blastocyst stage. To our knowledge, this is the first report in the literature showing the expression of the CTCF in preimplantation embryos and the presence of this gene in bovines. Further studies are needed to identify the complete sequence of the bovine CTCF gene. To elucidate the biological importance of CTCF and its role in human diseases and normal development, it is important to study homologous genes in other animals. It is possible to culture large proportions of bovine preimplantation embryos; besides, it represents an important model for the investigation of fetal development. Supported by: FAPESP, CAPES, CNPq, FAEPA.

Gene Expression Studies of Embryonic Stem Cells Using RNA Amplification and Microarray Technologies. *T.D. Gallardo*¹, *R.E. Hammer*^{2,3}, *D.J. Garry*¹. 1) Dept Internal Medicine, Univ Texas SW Medical Ctr, Dallas, TX; 2) Dept Biochemistry, Univ Texas SW Medical Ctr, Dallas, TX; 3) Howard Hughes Medical Institute, Univ Texas SW Medical Ctr, Dallas TX.

Gene expression profiles of thousands of genes can now be examined *en masse* through cDNA and oligonucleotide microarrays. Many studies have reported gene expression changes in cell lines, primary cells and tissues. However, present applications of microarray technology do not include the study of gene expression from minimal numbers of cells or individual cell types residing in a given tissue. Such advances would greatly facilitate our understanding of complex interactions between neighboring cell populations. We demonstrate here that gene expression studies can be successfully performed using a T7-based RNA amplification method combined with microarray technology. Using this strategy we have successfully amplified RNA from 100 embryonic stem cells for analysis of gene expression using Affymetrix oligonucleotide microarrays. RNA was isolated from a range of 100 to 100,000 stem cells, submitted through two rounds of amplification and then compared by array analysis to a set of unamplified samples. We observed greater than 91% of the transcripts expressed were changed less than two-fold. We have performed each amplification in triplicate and found this method to be not only highly reproducible but also sensitive for low abundance transcripts. Using this strategy we have begun to define the genetic program of the SM-1 embryonic stem cell population and have identified a number of novel genes and previously characterized genes to be expressed in this population (Oct-4, SSEA-1, AlkPhos., GCNF, etc). We believe the utilization of these combinatorial technologies will enhance our understanding of the cellular and genetic programs associated with these stem cell populations.

Roles for growth differentiation factor 3 in energy metabolism. *C.W. Brown¹, S.D. Coleman¹, D.E. Houston-Hawkins¹, M.M. Matzuk²*. 1) Depts of Molecular and Human Genetics and Pediatrics; 2) Depts of Pathology, Molecular and Cellular Biology and Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Growth Differentiation Factor 3 (GDF-3) is a secreted protein that is a member of the transforming growth factor beta superfamily. It shares significant homology with the *Xenopus laevis* mesoderm-inducing factor, Vg-1. PCR analyses of tissues from the adult mouse demonstrated expression of GDF-3 in multiple sites, including adipocytes. To better understand the functional role of GDF-3, we created mice with a homozygous null mutation at the GDF-3 locus. We examined the potential role of GDF-3 in the adipocyte by analyzing energy metabolism in the knockout mice. Although only modest differences in body weight were observed between the knockout mice and controls maintained on regular laboratory chow, dramatic differences in weight gain were observed when the mice were given high fat diets, despite similar caloric intake. Control male and female mice and homozygous mutant males became obese on this regimen, whereas homozygous mutant females maintained normal weight curves. Although control mice developed glucose intolerance on the high fat regimen, GDF-3^{-/-} females maintained essentially normal glucose tolerance curves. These findings suggest potential important roles for GDF-3 in maintaining normal body fat composition and glucose homeostasis.

Knock-in mouse models for phenotypic comparison of the Apert syndrome Fgfr2 Ser252Trp and Pro253Arg mutations. *R. Xiao¹, D. Huso², C.P. Lerner³, W.A. Paznekas¹, J. Conover³, K. Yu⁴, D.M. Ornitz⁴, E.W. Jabs¹.* 1) Inst. Genet. Med., Johns Hopkins Univ., Balto., MD; 2) Dept. Comparative Med., Johns Hopkins Univ., Balto., MD; 3) The Jackson Lab., Bar Harbor, ME; 4) Dept. Mol. Biol. and Pharmacology, Washington Univ., St. Louis, MO.

Apert syndrome is an autosomal dominant disorder characterized by craniosynostosis, cleft palate, multiorgan system malformations with severe and symmetric syndactyly. The phenotype results from mutations in fibroblast growth factor receptor 2 (FGFR2). Two thirds of the patients have the Ser252Trp mutation while the other third have the Pro253Arg mutation. These gain of function mutations cause a change in the fibroblast growth factor ligand binding affinity and specificity. In limited human studies, it has been suggested that craniofacial abnormalities such as cleft palate are more severe with the Ser252Trp mutation while limb malformations are more severe with the Pro253Arg mutation. To understand the pathogenesis of this developmental disorder and to delineate phenotype-genotype correlations, we generated two mouse models with mutations at the conserved Ser or Pro residue of the mouse sequence. Gene targeting by homologous recombination followed by Cre/loxP-mediated recombination was performed. Ser252Trp heterozygous mutant mice had smaller body size, abnormal head shape, and bulging eyes. There was a high incidence of perinatal lethality. Mutant mice that died within 24-36 hours of birth showed multiple abnormalities. The lung lesions had lack of alveolar expansion and diffuse atelectasis with accumulation of proteinaceous fluid in the alveoli and mild dilation of the bronchioles. Cardiovascular abnormalities included dilation of the atria and great vessels at the base of the heart. There was diffuse, severe lymphoid necrosis and apoptosis in the thymus and mild luminal ectasia was found in the stomach and the intestine. In addition, mutant mice had structural abnormalities of the palate. Pro253Arg heterozygous mutant mice were observed to have syndactyly. These findings are consistent with some of the major congenital abnormalities described in patients with Apert syndrome.

Sonic hedgehog induces ectopic expression of zebrafish iroquois gene (ziro1b) during neural development. C.H.M. Yan, S.H. Cheng. Biology and Chemistry, City University of Hong Kong, Hong Kong, Hong Kong.

Vertebrate embryo development relies on complex molecular mechanisms to ensure correct spatial and temporal gene regulations for the production of orderly developed tissues and organs. During the development of the vertebrate brain, proneural and neurogenic genes are required for proper specification of neuronal precursors. The *iroquois* family of homeobox genes, first isolated in *Drosophila*, has been found to act as pre-pattern factors involved in patterning and regionalization of embryonic tissues in both vertebrates and invertebrates. The expression of *iroquois* genes is maintained by *hedgehog* (*hh*) signaling and its downstream mediator, *Cubitus interruptus*, in some domains of *Drosophila*, while such relationship is largely unknown in vertebrate systems. The Hh family of secreted signaling proteins has been found to be responsible for developmental patterning in a variety of systems such as the neural tube. In this study, zebrafish was chosen as the vertebrate model organism to investigate the regulatory mechanism of a member of *iroquois* genes (*ziro1b*) by *sonic hedgehog* (*shh*) in neural development. The expression of *shh* was manipulated by RNA injection into fertilized embryos. *In situ* hybridization analyses were carried out on injected embryos at various time points. Overexpression of *shh* induced ectopic expression of *ziro1b* gene, which is normally expressed in the midbrain and hindbrain of developing embryos. This suggests that *shh* may regulate *ziro1b* in neural development.

Defective SRY phosphorylation and DNA-binding ability caused by a non-HMG box SRY mutation in a family presenting variable sex-reversed phenotypes. *J.G. Assumpcao¹, C.E. Benedetti², A.T. Maciel-Guerra³, G. Guerra-Jr³, M.R. Scolfaro³, M.P. De Mello¹.* 1) CBMEG, Univ Estadual de Campinas, Campinas, So Paulo, Brazil; 2) Centro de Biologia Molecular Estrutural - Laboratrio Nacional de Luz Sncroton (LNLS), Campinas, SP, Brazil; 3) 3Grupo Interdisciplinar de Estudos de Distrbios da Diferenciaio do Sexo (GIEDDS) - Faculdade de Cincias Mdicas, Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brazil.

The *SRY* gene (sex-determining region of the Y chromosome) initiates the process of male sex differentiation in mammals. We describe here a novel missense mutation in the *SRY* gene, a G to T transversion, which changes an arginine to an isoleucine at codon 30 (R30I). This mutation was found in affected and non-affected members of a family, including the father, two siblings with partial gonadal dysgenesis, a phenotypic female with pure gonadal dysgenesis and three non-affected male siblings. The G to T base change was not found in the *SRY* sequence of 100 normal males screened by ASO-PCR. The R30I mutation is located upstream to the HMG box, within the ²⁹RRSSS³³ PKA phosphorylation site. We demonstrate that the phosphorylation of the SRY^{R30I} protein in vitro is negligible. We also show that the non-phosphorylated SRY^{R30I} protein is able to bind the target DNA as effectively as the wild-type SRY; however, when using phosphorylated proteins in gel mobility shift assays, the SRY^{R30I} protein exhibits reduced DNA-binding activity in comparison to the phosphorylated wild-type protein. Therefore, we suggest that the activity of the SRY^{R30I} is close to a threshold level in which its ability to induce testicular development in vivo would depend on the physiological conditions or interactions with other genetic factors. This could explain the three phenotypes (normal male, partial gonadal dysgenesis and pure gonadal dysgenesis) observed in the individuals carrying the R30I substitution. Financial Support: CNPq, FAEP-UNICAMP.

Mutations in *GDF1* are associated with congenital cardiovascular malformations in humans. *J.B. Mez¹, J.D. Karkera¹, E. Roessler¹, E. Goldmuntz², P. Bowers³, J.A. Towbin⁴, J. Belmont⁴, S.C. Ekker⁵, M. Muenke¹.* 1) Medical Genetics Branch, NHGRI/NIH, Bethesda, MD; 2) Division of Cardiology, Children's Hospital of Philadelphia and the University of Pennsylvania School of Medicine, Philadelphia, PA; 3) Department of Pediatrics, Yale University School of Medicine, New Haven, CT; 4) Pediatric Cardiology, Baylor College of Medicine, Houston, TX; 5) Arnold and Mabel Beckman Center for Transposon Research, Institute of Human Genetics, Department of Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, MN.

The transforming growth factor- β (TGF β) superfamily is a large group of structurally related, proteolytically cleaved polypeptides, that regulate the formation of the primary germ layers and the establishment of the body axes during gastrulation. Several (TGF β) family members (*Nodal*, *Lefty-1*, *Lefty-2*, and *Growth/differentiation factor-1 (Gdf1)*) in the Nodal signaling pathway have been implicated in left-right (L-R) patterning in the mouse. *Gdf1*^{-/-} mice show complex and variable cardiac malformations due to L-R axis anomalies including randomization of the great arteries, atrial and ventricular septal defects, and atrioventricular (A-V) canal. To test whether this gene is involved in L-R asymmetry in humans, we characterized the human *GDF1* gene and screened 378 patients with congenital cardiac anomalies including truncus arteriosus, tetralogy of Fallot, transposition of the great arteries, double outlet right ventricle, A-V canal, or interrupted aortic arch for mutations in *GDF1*. We identified the following changes that were apparently unique to patients with congenital cardiovascular malformations (and not present in 200 chromosomes from normal controls): one nonsense mutation, seven missense mutations including an invariant cysteine knot, three silent mutations, a variation in the 5' untranslated region and four intronic variations. To better understand the underlying mechanisms of *GDF1* mutations as one cause for cardiovascular anomalies in humans, functional studies in zebrafish are in progress utilizing morpholino-induced *Vg1* zebrafish mutants.

Nicotine and embryonic development in zebrafish. *S. Beiraghi, E. Beckman, D. Shiroma, S.C. Ekker.* The Arnold & Mabel Beckman Center for Transposon Research, University of Minnesota, Minneapolis, MN.

Children of mothers that smoke have an increased risk for a number of developmental abnormalities including low birth weight, craniofacial anomalies such as cleft lip and palate. Nicotine can exert multiple effects on early embryogenesis, including altered patterns of cellular mitosis, cell migration, and other gross developmental defects in mice. The zebrafish (*Danio rerio*) offers many advantages to the study of this problem because of external embryonic development and the multiple genetic tools now available to this model organism. In addition, there is substantial similarity between the early development of the zebrafish and mammals, including mechanisms that underlie craniofacial or skeletal structure, making this a significant vertebrate system to explore the early developmental defects due to teratogens or mutagens. Objective: To investigate the effects of L-nicotine on zebrafish during embryonic development. Methods: We performed a dose response profile and determined an LD50 (0.025%) and ED (0.0031%) of L-nicotine. These embryos displayed wide spectrum developmental abnormalities as determined using standard microscopy approaches for morphological criteria. Experimental group were bathed for 24 hours in L-nicotine with different concentration (0.025 %-0.0031%), and the control group were bathed in egg-water for the same duration. Three markers (OTX-2, MYO-D, GATA-1) was used in in situ hybridization to investigate the patterns of expression of anterior ectodermal, somitic mesodermal, and ventral mesodermal tissue derivatives. Alcian blue staining was used on 5 day larvae to follow development of the jaw. Results: Nicotine exposure in higher concentration (0.025%) resulted in embryos with a wide spectrum of severe malformations with an underdeveloped tail and jaw and a reduced number of somites, and subsequent complications in jaw formation. As the concentration of nicotine reduced, the anomalies were less severe, however, subtle physiological changes such as; slow heart rate in experimental groups. Conclusion: Our preliminary data supports the view that nicotine is a teratogen, particularly affecting early vertebrate embryogenesis.

Sequence variations in the dystrophin gene, biological consequences, and clinical correlations. *C.S. Richards¹, Y.P. Weng¹, D. Odenbaugh¹, G. Miller¹, D.R. Love², M.R. Hegde¹.* 1) Dept Molec & Human Genet, Baylor Col Med, Houston, TX; 2) School of Biological Sciences, University of Auckland, Auckland, New Zealand.

Duchenne/Becker muscular dystrophy (DMD/BMD) is an X-linked recessive neuromuscular disorder affecting approximately 1 in 3500 males with one-third of isolated cases resulting from a new mutation in the dystrophin gene. The dystrophin gene, located on Xp21 is greater than two megabases and encompasses 79 exons. Two-thirds of patients have structural mutations with approximately 60% having intragenic deletion mutations and 6% duplication mutations, which can be detected using multiplex PCR analysis and/or Southern blotting. The remaining one-third of the patients carry non-deletion/duplication mutations that escape detection by routine genetic testing performed in diagnostic molecular laboratories. These non-deletion/duplication mutations in sporadic cases are problematic in risk analysis for at-risk females, particularly the mother of the affected child. Thus, prenatal diagnosis in these types of families is inaccurate and problematic. We have developed an assay, which addresses this serious and immediate need. We have developed a comprehensive mutation analysis strategy for the entire dystrophin gene to identify not only whole exon deletion/duplication mutations, but also point mutations, small deletions and small insertions. The strategy involves amplification of all exons of dystrophin gene and analysis of each exon using denaturing high performance liquid chromatography (dHPLC). Missense mutations represent challenges in sequence interpretation and are often reported as unclassified variants. One strategy which may help resolve such interpretation dilemmas is the use of protein modeling. We intend to characterize each missense mutation identified in the dystrophin gene by sequence analysis and map it to the corresponding dystrophin protein domain and perform protein modeling. A better characterization of all sequence variations within the dystrophin gene will lead to a better understanding of the functional domains of the dystrophin gene and the relationship to disease phenotype.

Myosin VI and hereditary hearing loss: from mouse to man and back to mouse. *K.B. Avraham¹, N. Ahituv¹, G. Gasparini², Y. Raphael³*. 1) Dept. of Human Genetics & Molecular Medicine, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel; 2) Genetica Medica, Dip. Patologia Generale, II Universite di Napoli and Tigem, Italy; 3) Kresge Hearing Research Institute, The University of Michigan, Ann Arbor, MI, USA.

The past few years have provided an explosion in our understanding of how the inner ear functions. This dramatic increase is due in large part to the genes found to be associated with nonsyndromic hearing loss. Since 1995, 27 genes have been found, and these have provided clues about auditory transduction, ion homeostasis, and inner ear development. In particular, mouse models for human deafness, with mutations in orthologous genes, have revealed essential information about the pathophysiology caused by these mutations. One group of proteins frequently associated with hearing loss are the myosins, three of which were discovered thanks to their corresponding mouse mutants. Myosins are molecular motors that move along actin filaments and have been implicated in various cellular functions such as cell movement, membrane traffic, and signal transduction. An intragenic deletion in myosin VI (*Myo6*) leads to deafness and vestibular dysfunction in Snell's waltzer mice. A genome scan on DNA derived from an Italian family with progressive dominant hearing loss revealed linkage to chromosome 6q13, the region containing the human *MYO6* gene (Melchionda *et al. AJHG* 2001). Subsequently, a missense mutation was found in the coding region of this gene, which replaces a cysteine with a tyrosine at residue 442 of the protein (C442Y). The following suggests that the deafness in this family is due to the *MYO6* missense mutation: the segregation of this mutation with the affected individuals in the family, the previous association of myosin VI with deafness, and the conservation of the mutated residue. We have now confirmed that this mutation is the cause of deafness in humans, since reproduction of this mutation in transgenic mice has revealed a similar progressive hearing loss. A morphological analysis of the transgenic mice demonstrates the pathophysiology of this mutation.

Eya4 and Its Interacting Partners. *Y. Zhang*¹, *S. Wayne*¹, *R.A. Friedman*², *G. Van Camp*³, *R.J.H. Smith*¹. 1) Molecular Otolaryngology Research Laboratories, University of Iowa, Iowa City, IA, USA; 2) House Ear Clinic and House Ear Institute, Los Angeles, CA, USA; 3) Department of Medical Genetics, University of Antwerp, Antwerp, Belgium.

Statement of Purpose. Our work identifying mutations in EYA4 in two families with DFNA10 implicates this gene as essential for normal auditory function. EYA genes, four of which are known in humans, are expressed in a wide range of tissues. The mutations we identified in EYA4 are predicted to affect the *eya* homologous region (*eyaHR*). Given the role of the *eyaHR* to EYA protein function, it is not surprising that these mutations have a phenotypic correlate. Neither is the association of late-onset hearing loss with transcriptional activators unprecedented, as mutations in POU4F3 are known to cause postlingual hearing loss at the DFNA15 locus (Vahava et al., 1998). What is surprising is the limited DFNA10 phenotype, especially when one considers the clinical impact of mutations in another EYA gene, EYA1. In this study, we determined the interacting partners of Eya4. **Methods Used.** We used RT-PCR to identify isoforms of Eya4 in total RNA isolated from multiple mouse tissues. To determine the normal functional partners of the Eya4 protein and to assess the impact of specific Eya4 mutations on these interactions, we used the yeast two-hybrid system and cell culture/transfection assays. **Summary of Results.** We identified three novel splice variants of Eya4 and demonstrated expression of Eya4 in multiple tissues. Based on these findings, we constructed several bait vectors by fusing select portions of the Eya4 coding sequence with a GAL4 DNA-binding domain; in addition, we made constructs that contain the two mutations associated with the DFNA10 phenotype. We used these constructs to test the interaction of Eya4 with prey constructs of Six1 and DACH, two proteins that are known to interact with Eya1 (Buller et al., 2001). To identify partners of Eya4 that are unique to the cochlea, we constructed a cochlear cDNA prey library. **Conclusion.** Eya1 and Eya4 show differing affinities for Six1 and DACH. Eya4 interacts with several cochlear-expressed proteins. (Supported by DC03544 to RJHS).

Mutation Screening of *TMC1* in DFNB7/11 Families. *T. Yang*¹, *R. Carmi*², *Y. Bayazit*³, *A. Ramesh*⁴, *K. Kurima*⁵, *B. Cable*¹, *K. Elbedour*², *C.R.S. Srisailapathy*⁴, *A.J. Griffith*⁵, *V.C. Sheffield*⁶, *R.J.H. Smith*¹. 1) Genetics Program, University of Iowa, Iowa City, IA, USA; 2) Genetic Institute, Soroka University Medical Center, Ben-Gurion University of Negev, Negev, Israel; 3) Gaziantep University, Department of Otolaryngology and Head and Neck Surgery, Kolejtepe, Gaziantep, Turkey; 4) Department of Genetics, University of Madras, Madras, India; 5) National Institute on Deafness and Other Communication Disorders, National Institutes of Health, Rockville, Maryland, USA; 6) Howard Hughes Medical Institute and Department of Pediatrics, University of Iowa, Iowa City, IA, USA.

Statement of Purpose. Autosomal recessive nonsyndromic deafness (ARNSD) is the most common form of congenital inherited deafness. A novel gene *TMC1* has been identified as being responsible for ARNSD at the DFNB7/11 locus. Mutations in this gene also cause autosomal dominant hearing loss at the DFNA36 locus (Kurima et al., 2002). We screened our DFNB7/11 families to determine whether mutations in *TMC1* were causally related to their hearing loss. **Methods Used.** Mutation screening of *TMC1* was completed using bi-directional sequencing. In addition, we used computational analysis of the genomic sequence that includes *TMC1* to predict additional potential exons. **Summary of Results.** We identified an R34X nonsense mutation in one Bedouin family and two unrelated Turkish families. In two other DFNB7/11 families, screening of the reported coding sequence of *TMC1* failed to reveal mutations that segregated with the deafness phenotype. This finding suggests the possibility of unrecognized or alternatively spliced *TMC1* exons that contribute to the DFNB7/11 mutation load. Sequencing of predicted exons has revealed a point mutation segregating with the deafness phenotype in an Indian family. **Conclusion.** Mutations at DFNB7/11 locus appear to be a relatively common cause of ARNSD. It is possible that there are novel splice variants of *TMC1* that are cochlear-expressed and causally related to deafness at this locus. (This research was supported by R01-DC02842 to RJHS.).

Spinocerebellar ataxia type 10 protein binds to Myosin phosphatase target subunit 1 (MYPT1). *S. Nagamitsu*¹, *R. Zhang*², *T. Matsuura*², *H.F Epstein*², *T. Ashizawa*². 1) Department of Pediatrics, Kurume University, Fukuoka, Fukuoka, Japan; 2) Department of Neurology, Baylor college of Medicine, Houston, TX.

Spinocerebellar ataxia type 10 (SCA10) is an autosomal dominant disorder caused by expansion of an unstable pentanucleotide ATTCT repeat in intron 9 of the *SCA10* gene. While the number of ATTCTs does not exceed 22 in normal subjects, affected individuals have ATTCT repeats ranging from 800 to 4,500 repeats. However, the mechanism by which the expansion mutation in this gene causes the disease phenotype is not understood. The *SCA10* gene encodes a novel protein known as E46L, which has no recognizable motifs or predicted structures. To gain insight into the function of this protein, the yeast two-hybrid system was utilized to identify proteins that interact with E46L. In the screening of 5 million yeast clones expressing human brain cDNA proteins, 25 clones were identified to interact with E46L; 9 of these 25 clones encoded myosin phosphatase target subunit 1 (MYPT1). This protein-protein interaction was confirmed by both in vitro protein binding assay and immunohistochemistry. E46L was localized in both nucleus and cytosol, and colocalized with MYPT1 mainly in the cytosol in the transfected PC12 cells. In vitro protein binding studies using truncated MYPT1 proteins suggested that E46L binds to the carboxyl terminal portion of MYPT1. Although this portion of MYPT1 includes phosphorylation sites, phosphorylation of MYPT1 was not affected by E46L. In transfected PC12 cells, over-expression of E46L with or without over-expression of MYPT1 did not cause any detectable morphological changes including neurite outgrowth. MYPT1 phosphatase activity on myosin light chain in the presence of E46L is currently investigated in our laboratory.

Potential Modifier Role of the R618Q Variant of Proa2(I)collagen in Type I Collagen Fibrillogenesis: In Vitro Assembly Analyses. *A.N. Vomund¹, S.R. Braddock², C.L. Phillips^{1,2}*. 1) Dept Biochemistry, Univ Missouri, Columbia, MO; 2) Dept Child Health/ Medical Genetics, Univ Missouri, Columbia, MO.

An arginine to glutamine substitution at amino acid position 618 in the triple helix of proa2(I)collagen (R618Q) was first reported in a patient with a variant of Marfan syndrome. This substitution was later identified in conjunction with a second mutation in a patient diagnosed with osteogenesis imperfecta. Yet, in both cases, R618Q was also present in unaffected or mildly affected family members, suggesting R618Q may represent a rare polymorphism. However, arginine₆₁₈ in the triple helix is conserved across a variety of species and fibrillar collagen types, suggesting it is functionally significant. We hypothesize that the R618Q allele of proa2(I)collagen may act as a modifier of connective tissue structure/function and is not a primary disease causing allele. To evaluate the functional significance of the R618Q allele, we isolated type I collagen from cultured dermal fibroblasts from control individuals and two unrelated individuals who were heterozygous for R618Q allele and evaluated triple helical stability and fibril assembly. We analyzed type I collagen helical stability by protease resistance under thermal denaturing conditions and by CD spectroscopy, and demonstrated that there were no statistical differences between control and R618Q containing collagen. We evaluated fibrillar assembly using an in vitro assembly assay, which demonstrated that fibrils containing R618Q collagen molecules exhibited more rapid fibril growth and no apparent fibril nucleation phase when compared with control in vitro assembled type I collagen fibrils. Further, electron microscopy analyses indicated that assembled R618Q fibrils have diameters, which are approximately 20 percent of in vitro assembled control type I collagen fibril diameters. These data suggest that R618Q containing collagen does not play a significant role in the triple helical stability of type I collagen, but points to a role for arginine₆₁₈ in fibril assembly, and supports the hypothesis that the R618Q variant may have a role in modifying connective tissue structure and function, and potentially in disease pathogenesis.

Pathogenic Molecular Signals Underlying Non-Union Skeletal Fractures. *K.E. Pearson¹, M. Bostrom¹, R. Hotchkiss², D.A. Stephan².* 1) Genetic Med, CNMC, Washington, DC; 2) Hosp. for Special Surgery, New York, NY.

Non-union skeletal fractures are characterized by their inability to heal six months after injury. Approximately 100,000 non-union fractures occur annually in the United States. Bone graft surgery is the most common form of treatment while other methods such as electrical stimulation are used to accelerate the natural bone healing process. Current therapies are extremely invasive, painful, and expensive. Left untreated, non-unions may cause advanced arthritis and loss of function in the affected limb. Here we aim to identify novel treatment options through a detailed understanding of the pathogenic process. Oligonucleotide arrays were used to identify the mechanisms that lead to lack of skeletal repair in non-unions. Biotynlated cRNA was generated from four normal fracture callus tissue samples and pooled and hybridized to two replicate Affymetrix chips. The same method was applied for five non-union callus samples. This pooling strategy physically normalizes inter-individual differences, allowing disease-specific correlates to be identified with a greater than 80% true positive ratio. Lists of upregulated and downregulated genes were produced to isolate genes with fold changes greater than two. The absence of genes responsible for the early inflammatory stage of healing may suggest the lack of infiltration of macrophages, monocytes, lymphocytes, and polymorphonuclear cells, which are responsible for the ingrowth of vascular tissue, formation of granulation tissue, and the migration of mesenchymal cells. Instead, non-union tissue is abundant in B-cell type-II response genes such as the various chains of immunoglobulins and B-lymphocyte chemoattractants, along with many extra-cellular matrix protein and skeletal muscle genes. Immunohistochemical staining of several unique non-union and normal frozen callus tissue samples was performed using antibodies against several upregulated and downregulated genes to confirm the array data. In vivo studies are underway. Our findings indicate that a lack of an inflammatory/immune response is the cause of non-union fractures in skeletal tissue. This insight allows directed therapeutics to be developed.

Mapping and identification of CRX - ataxin-7 binding domains and a novel ataxin-7 nuclear localization domain.

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Spinocerebellar ataxia type 7 (SCA7) is caused by a CAG repeat expansion. We recently showed that polyglutamine-expanded ataxin-7 directly interacts with cone-rod homeobox (CRX) protein and that SCA7 retinal degeneration may involve interference with CRX function. To characterize the CRX-ataxin-7 interaction, we generated CRX deletion constructs. In vitro co-ip studies using different N-terminal deletions established that the glutamine-rich and homeodomain regions of CRX are sufficient for interaction with ataxin-7. While deletion of the seven C-terminal glutamines (Q's) from the glutamine-rich domain diminished but did not eliminate the interaction, non-conservative mutagenesis of the two N-terminal homeodomain Q residues in this context abolished the ataxin-7 interaction. To evaluate ataxin-7, we generated N-terminal and C-terminal deletions of ataxin-7 with 92 Q's, and tested the ability of the ataxin-7 deletion proteins to repress CRX transactivation of a rhodopsin promoter-reporter construct in HEK293 cells. Importantly, C-terminal deletions of ataxin-7 92Q markedly reduced its ability to repress CRX transactivation. Analysis of subcellular localization of C-terminal deletions suggested that they were predominantly cytoplasmic, and thus unable to enter the nucleus and interfere with CRX function. When ataxin-7 92Q was truncated to a 147 aa N-terminal protein, nuclear localization became prominent, likely due to its small size, and was accompanied by a return of full repression. Analysis of the ability of ataxin-7 92Q N-terminal deletions to repress CRX-mediated transcription revealed that the Q tract of ataxin-7 is required for inhibition. Interestingly, loss of the first 25 amino acids of ataxin-7 significantly reduced the level of CRX interference. This finding suggests that the most N-terminal ataxin-7 amino acids either participate directly in the CRX interaction or that their loss alters the tertiary structure of ataxin-7 or its Q tract such that interaction with CRX is diminished.

The transcription factor SOX10 regulates PLP expression in the central nervous system. *M. Girard¹, N. Bondurand¹, N. Lemort¹, V. Pingault^{1,2}, M. Goossens^{1,2}.* 1) Genetics, INSERM U468, Creteil, France; 2) Laboratoire de Biochimie et Genetique Moleculaire, AP-HP, Hospital Henri Mondor, Creteil, France.

SOX10 is an essential factor in the enteric nervous system (ENS), melanocytes and glial cells development. Mutations in the SOX10 gene have been described in several cases of Shah-Waardenburg syndrome, a neurocristopathy characterized by the association of Hirschsprung disease (intestinal aganglionosis) and Waardenburg syndrome (pigmentation defects and sensorineural deafness). In accordance, it was shown that SOX10 controls expression of MITF and RET, which play important roles during melanocytes and ENS development, respectively. Some patients also present with myelination defects of the peripheral nervous system (PNS), which is in agreement with the demonstration that P0 and Cx32, two major proteins of the PNS, are controlled by SOX10. Nevertheless, these findings cannot explain the defects of the central nervous system (CNS), consistent with Pelizaeus-Merzbacher disease (PMD), observed in one patient. This suggests that SOX10 may regulate other genes involved in the myelination process of the CNS. To test this hypothesis, we sought the possible involvement of SOX10 in the regulation of expression of PLP and its alternative transcript DM20. Both proteins are major components of myelin in the CNS, and mutations of the PLP gene are associated with PMD. Here we show that SOX10 activates expression of the PLP gene in transfection assays, while SOX10 mutant proteins fail to transactivate this promoter, and that EGR2, another major regulator in the CNS, is also able to regulate the PLP promoter. These results were further confirmed by the study of a cell line expressing SOX10 in an inducible manner, where PLP and DM20 expression is upregulated when SOX10 is induced. Overall, our results provide insights into the regulation of PLP expression, and underline the importance of SOX10 during the myelination process. Our study may also improve our understanding of molecular mechanisms involved in the phenotypic features of some peripheral neuropathies.

Repair capacity of motor neurons in spinal muscular atrophy. *T. FRUGIER, R. OLASO, N. ROBLLOT, V. JOSHI, J. MELKI.* Molecular Neurogenetics Laboratory, INSERM E.9913, Evry University, GENOPOLE, Evry, France.

Mutations of the survival of motor neuron gene (SMN1) are responsible for spinal muscular atrophy (SMA), a common genetic cause of death in childhood. SMA is characterized by degeneration of motor neurons leading to progressive paralysis with muscular atrophy. The Cre-loxP recombination system has been used to direct deletion of the murine *Smn* exon 7 to neuronal tissue. Mutant mice display severe muscle paralysis leading to death within the first 4 weeks of age (Frugier et al., 2000). To evaluate the repair capacity of motor neurons, conditional expression of an intact human SMN cDNA at various times of the disease course has been carried by using the tetracycline-controlled transactivator (tTA system). A transgenic line expressing hSMN cDNA placed under the control of the tetO-CMV promoter (tetO-hSMN) has been generated and crossed to transgenic line expressing tTA under the control of the prion gene promoter (Prp-tTA, Tremblay et al., 1998). An induction or repression of the hSMN transgene expression has been observed in response to the absence or presence of doxycycline treatment respectively. We are currently crossing the appropriate progenitors to produce mice carrying Prp-tTA, tetO-hSMN transgenes and the deletion of SMN in neurons. Doxycycline will be stopped at various times of the disease course. We will evaluate the expression pattern of hSMN mRNA and protein and will examine the effect of hSMN transgene expression on SMA phenotype. In addition, elucidating the molecular mechanism involved in or associated with motor neuron repair through gene expression profiling studies should contribute to identify new therapeutic targets in SMA and possibly in other degenerative disorders.

Characterization of Torsin Related Proteins (Tor2A/Tor3A). *M.M. Caton¹, S. Jose¹, P. De Carvalho Aguiar¹, J. Hewett², X.O. Breakefield², L. Ozelius¹.* 1) Department of Molecular Genetics, Albert Einstein College of Med.; 2) Molecular Neurogenetics, Massachusetts General Hospital Charlestown, Ma.

Dystonia is a movement disorder, characterized by involuntary twisting movements and abnormal postures. Over 13 different genes have been implicated in various forms of primary, hereditary dystonia. The DYT 1 locus on human chromosome 9q34 is responsible for early onset dystonia (EOD). Inheritance of this gene is autosomal dominant with reduced penetrance (30-40%). DYT1 is a member of a gene family consisting of several other highly homologous genes. Two members, TOR2A and TOR3A/ADIR1 share about 45% homology at the amino acid level with DYT1. We are interested in characterizing TOR2A and TOR3A, as these genes are potential candidates for involvement in other forms of dystonia, could influence the penetrance of EOD, or may provide clues to the function(s) of the DYT1 gene. Northern analysis showed that both messages are widely expressed in peripheral tissues, as well as in various regions of the brain. Genomic sequence analysis showed that TOR3A mapped to chromosome 1 and TOR2A to chromosome 9. Analysis of exon:intron structure revealed that both genes are structurally similar to DYT1. Further EST database searches revealed that there are several isoforms of TOR2A, which are derived by alternative splicing. In order to determine whether these isoforms are present in cDNA of adult brain, we performed PCR amplification of Tor2a. We have identified two alternative splice variants containing open reading frames, the first encodes exons 2, 3, 4, 5, and the second, exons 2, 3, 5. In order to determine the cellular localization of TOR2A and 3A, the full-length cDNAs were cloned into expression vectors and transfected into 293T cells. Immunofluorescence staining showed that both proteins co-localize with antibodies for Protein Disulfide Isomerase (PDI) suggesting that similar to torsinA, Tor2a and 3a are resident in the endoplasmic reticulum. Finally, we are in the process of examining genetic markers located close to these genes on chromosome 1 and 9 in several large families with dystonia not linked to other known loci.

Functional characterization of genetic polymorphisms in the human serotonin receptor 1B (HTR1B) gene promoter. *J. Duan, A.R. Sanders, P.V. Gejman.* Schizophrenia Genetics Research Program; The University of Chicago; Chicago, IL.

HTR1B has been suggested to play an important role in multiple psychiatric disorders and conditions, and therefore many association tests have been published with varied results. Hence, our group initiated a systematic study of the functional correlations of genetic diversity in HTR1B and are reporting now the effect on transcription of polymorphisms located at the *in silico* predicted HTR1B promoter sequences. Using the TSSG program for recognition of human PolIII promoter region and start of transcription, we determined that a 5 segment (-1 to 1387) contains a putative functional promoter spanning a TATA box (TATAAA) from -911 to 906. This 5 segment also spans common variations, T-261G, A-161T, and -182INS/DEL-181 (insertion/deletion spanning 2 bp). We have used a SEAP (secreted alkaline phosphatase) reporter gene system to measure the transcription activity. We found that the putative promoter segment exhibited transcriptional activity after 96 hr of transfection in HCN-1A (human cortical neuron) and 48 hr of transfection in CHO-K1 (Chinese hamster ovary) cells. Interestingly, the haplotype -261G/-182INS-181/A-161 showed a 2-fold enhanced transcriptional activity compared with the haplotype T-261/-182INS-181/A-161. The effect was not detected with -261G/-182INS-181/-161T, which suggests that the -161T allele has a compensating negative effect on transcription. The TSSG program also predicted that A-161T and -182INS/DEL-181 fall into putative transcription factor (TF)-binding sites, and that the -261G allele generates a new TF-binding site (CCCMNSSS). To assess whether the functional effect of the alleles -261G and -161T could be explained by the modification of a specific binding to TF, we performed an electrophoretic mobility shift assay. The oligonucleotide probe spanning allele -261G showed specific binding to an unknown TF from nuclear extracts, but no specific binding was detected for T-261 allele. These results provide an explanation for the increase of the transcription activity of the promoter. Our experiments have relevance to the design and interpretation of association studies with HTR1B.

Gene structure and protein function studies for Sacsin. C. LeBlanc¹, J. Mercier¹, P. Lepage², T.J. Hudson², A. Richter¹. 1) Génétique Médicale, Hôp. Sainte-Justine; 2) Montreal Genome Centre, McGill Univ. Health Ctr.; Montreal, Canada.

Mutations in *sacsin* (g.6594delT (DT) and g.5254C®T, (C®T)) are responsible for autosomal recessive spastic ataxia of Charlevoix-Saguenay (C-S), (ARSACS/SACS MIM# 270550), a clinically homogeneous early onset disease. Different disease causing mutations have been described in other populations. Gene predictions of the Genome Project Working Draft at <http://genome.ucsc.edu/> suggest that coding regions 5' of the *sacsin* ORF are also part of the gene. Using 5' RACE and RT-PCR analyses combined with direct sequencing we determined that 9 other smaller exons likely are part of *sacsin*, making the genomic region close to 105 kbases. Experiments are underway to define the still elusive transcription start site. We can detect over 97% of disease alleles in C-S ARSACS patients. In 5 other patients with classical ARSACS phenotype from the C-S region, one disease allele is still undetected. We are now scanning the upstream exons in these patients for mutations.

To investigate the function of the *sacsin* protein we raised polyclonal Abs to two cloned polyHis-tagged *sacsin* fragments produced in a bacterial expression system. Western blotting of total protein from mouse brain and liver using both Abs revealed the presence of a >427 kDa protein band in brain that was absent in liver. The MW was estimated in comparison to dystrophin, detected by a monoclonal anti-dystrophin Ab. Western analysis of protein extracts from fibroblasts of ARSACS patients with DT/DT, DT/C®T and C®T/ C®T genotypes and controls showed the specific absence of the high molecular weight protein band. Immunocytochemistry using Ab to the C terminal portion of *sacsin* on control and patient fibroblasts shows reduced cytoplasmic localization of *sacsin* in patients. The intense granular staining with Ab to a more NH₂ terminal region suggests the presence of truncated *sacsin* in patient cells. Cell fractionation and immunohistochemistry with patient and control tissues are in progress. **Supported by the Canadian Institutes of Health Research.**

The expression of polyglutamine and $\alpha 1A$ -voltage-dependent calcium channel protein in the SCA6 brain. *K. Ishikawa, K. Ishida, H. Fujigasaki, K. Owada, H. Mizusawa.* Dept Neurology, Tokyo Medical & Dental Univ, Tokyo, Japan.

Spinocerebellar ataxia type 6 (SCA6) is a dominantly-inherited degenerative disease caused by a small expansion of the CAG repeat that encode polyglutamine tract in the $\alpha 1A$ -voltage-dependent calcium channel gene. By immunohistochemistry, two types of microscopic aggregations composed either of $\alpha 1A$ -calcium channel protein or polyglutamine have been identified in the cytoplasm of the cerebellar Purkinje cells that are predominantly affected in this disease. Importantly, these two types of aggregates are so far specific to SCA6 Purkinje cells, and have been shown not co-localize each other. To elucidate the precise nature of these aggregates, we examined frozen brain tissues of control human and SCA6 patients on western blot using antibodies against $\alpha 1A$ -calcium channel, calcium-binding protein calbindin-D28k, and expanded-polyglutamine. The expression of $\alpha 1A$ -calcium channel protein was demonstrated in the cerebral cortex, basal ganglia, hippocampus and the cerebellar cortex of both control subjects and patients with SCA6. On western blot, the expression level of the channel protein relative to that of calbindin-D28k, a marker for Purkinje cells in the cerebellum, was not obviously decreased in the SCA6 cerebellum. No aberrant band was detected in lysates from SCA6 brain tissues. Immunohistochemical staining also showed that immunoreactivity for anti-calcium channel antibody is not altered in Purkinje cell soma, nor in the molecular and granule cell layers of SCA6 cerebellum. Western blot for expanded-polyglutamine failed to detect any aberrant bands from the SCA6 brain tissue. The present study indicates that the small polyglutamine expansion do not alter the expression level of $\alpha 1A$ -calcium channel protein. The mechanism underlying the formation of polyglutamine aggregates in SCA6, which are mainly seen in the cytoplasm of Purkinje cells, may not be identical to that of polyglutamine aggregates in other polyglutamine diseases.

CLN3 modulates cell growth and apoptosis. *D.N. Persaud-Sawin¹, A. Van Dongen^{1,3}, R-M. Boustany^{1,2}.* 1) University Program in Genetics, Duke University, Durham, NC; 2) Department of Pediatrics, Duke University, Durham, NC; 3) Department of Pharmacology, Duke University, Durham, NC.

Juvenile Batten disease (JNCL) is an autosomal recessive disease that results from mutations in the CLN3 gene. The CLN3 gene coding sequence has 15 exons, and the translated protein consists of 438 amino acids. The most commonly observed mutation is a 1.02 Kb deletion in the genomic DNA. This deletion results in a truncated protein of 24KDa in size. This is due to the loss of amino acids 154-438, and the introduction of 28 novel amino acids at the carboxyl end. We demonstrate that compared to normal controls, CLN3 deficient lymphoblasts homozygous for this common deletion grow slower and show increased sensitivity to etoposide-induced apoptosis, supporting the notion that CLN3 may negatively regulate apoptosis. Using these JNCL lymphoblasts as a model system, we assess the effects of specific CLN3 mutations on cell growth and protection from etoposide-induced apoptosis. Protection from etoposide-induced apoptosis occurs and the observed growth deficiency is corrected, following transfection of these JNCL lymphoblasts with mutant CLN3 cDNA that includes exons 11 or 13. We show that deletion of the conserved region 184WSSGTGGAGLLG195, creates a growth phenotype similar to the JNCL patient cells. However, individual mutations within this region do not by themselves create diminished growth. We also show that mutations within glycosylation sites 71NQSH74 and 310NTSL313 and within the highly conserved amino acid stretches 291VYFAE295 and 330VFASRSSL337, result in slowed growth and susceptibility to apoptosis. These findings suggest that 1) glycosylation at the above two sites affects the impact of CLN3 on cell growth and apoptosis; and 2) the highly conserved stretches VYFAE and VFASRSSL may represent motifs essential for preserving the positive effect of CLN3 on cell growth and the negative regulation of apoptosis. This information may aid in the design of drugs or therapies for JNCL.

Negative Regulation of Tyrosine Hydroxylase Expression by the Tumor Suppressor PTEN. *J.M. Roberts¹, S.A. Moussatov², M. Sugiultzoglul¹, M.G. Kaplitt^{1,2}.* 1) Neurosurgery, Weill Med Col, Cornell Univ, New York, NY; 2) Lab. of Neurobiology and Behavior, The Rockefeller Univ., New York, NY.

PTEN is a tumor suppressor gene which is frequently mutated in a variety of cancers including high grade gliomas. Although wild-type PTEN is known to modulate cellular responses to growth factors by opposing the ERK/MAPK and PI3K/Akt pathways, its function in regulating gene expression is far less understood, particularly in non-neoplastic cells. While studying potential effects of PTEN on neuronal signal transduction, we unexpectedly discovered tyrosine hydroxylase (TH) expression to be profoundly down-regulated. PC12 cells are a rat pheochromocytoma line which has been widely used to study expression of neuronal genes, including TH, which is the rate-limiting enzyme in the synthesis of dopamine. As analyzed by western blot, stable PTEN overexpression in PC12 cells reduced TH protein levels by at least 20 fold. Not only was this effect consistent among several independent clonal isolates, it also appeared to be specific for TH expression, since several other house-keeping genes tested were not affected. In comparison to control cells, PTEN over-expressing cells revealed approximately a 100-fold decrease in TH mRNA as determined by quantitative PCR. This suggests that PTEN elicits its effects on TH protein expression mostly by modulating mRNA levels. We are currently conducting studies to identify potential elements in the rat TH promoter which may mediate this effect in PTEN-overexpressing cells. By affecting dopamine synthesis in neurons, this tumor suppressor may also serve to modulate neurotransmission. Thus, our findings suggest that the role of PTEN is more complex than has been previously believed.

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Alternative transcripts of human brain-specific genes *MOB* and *CPLX 2*. *N.M. Raevskaya, I.P. Vladychenskaya, L.V. Dergunova, S.A. Limborska.* Human Molecular Genetics Dept, Inst. of Molecular Genetics, Moscow, Russian Federation.

Working on the project of the human brain-specific genes investigation we have obtained new in vitro and in silico data on structure and function of the human genes *MOB* and *CPLX 2*. Novel human brain-specific gene *MOB* coding the hypothetical transmembrane protein and located on the human chromosome 10 was demonstrated to span more than 219 kb. BLAST analysis supported by the results of RT-PCR has shown at least 11 exons and 10 introns to represent the genomic structure of *MOB* gene. Two alternatively spliced mRNA transcripts differing in their 5'-ends and possessing the same 3'-end were recognized in the human cerebellum mRNA samples. The length of the shorter transcript is estimated of 3,2 kb; 5'-end for this transcript is detected by means of RACE PCR. In *CPLX 2* gene, BLAST analysis has proposed the existence of an additional exon located within the intron 2 sequence at the distance of about 7 kb from the exon III. The resulting alternative transcript is thought to differ with its 5'-end from that for the previously described transcript. Alternative transcripts of the human complexin 2 gene differing with their 5'-ends encode the same protein product. We confirmed the existence of the predicted transcript using RT-PCR technique.

Gene Expression in Rhesus Monkey Brain. *B. Su*^{1,2}, *Y. Li*¹, *Y. Ma*³, *X. Zhang*³, *W. Huang*⁴, *L. Jin*^{2,4}. 1) 1.Key Laboratory of Cellular and Molecular Evolution, Kunming Institute of Zoology, the Chinese Academy of Sciences, Kunming, China; 2) 2.Center for Genome Information, Department of Environmental Health, University of Cincinnati, USA; 3) 3.Department of Neuroscience, Kunming Institute of Zoology, the Chinese Academy of Sciences, Kunming, China; 4) 4.National Genome Center at Shanghai, China.

Brain enlargement with improved cognitive skills is the most significant change during primate evolution and origin of our own species. It is widely believed that the prefrontal cortex (PFC) is the region in primate brains responsible for complex cognitive skills. In this study, we constructed a cDNA library from the PFC of a male rhesus monkey brain. The cDNA library contains about one million cDNA clones with insert size ranging from 500bp to 2kb. Our preliminary screening of sequencing 2,000 cDNA clones identified about 150 genes expressed in the rhesus monkey PFC with most of them also expressed in human brain according to previous studies, suggesting similar set of genes expressed both in human and rhesus monkey brains. The sequence similarity of the genes ranges from 90% to 97% between human and rhesus monkey confirming the suggested coding region sequence divergence between these two species.

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The Induced Mutant Resource: New tools for neurological research. *S.F. Rockwood, L.E. Mobraaten, M.T. Davisson.* Induced Mutant Resource, Jackson Laboratory, Bar Harbor, ME.

Transgenic and targeted mutant mice have proven to be useful in a wide variety of neurological research applications. The Induced Mutant Resource (IMR) at The Jackson Laboratory was formed in 1992 to serve as a centralized facility for the collection and distribution of induced mutant mice to the scientific community. The IMR distributes a wider variety of induced mutants than any other repository, with neurological mutants representing approximately 20% of available stocks. Neurological mutants fall into one or more of four categories. *Basic Mutations* that don't represent specific disease models, but are useful in delineating the biological role of specific genes, represent the bulk of the IMR's neurological mutant collection. *Disease Models* that recapitulate aspects of Alzheimer's, Huntington's, Parkinson's and other diseases provide useful tools for the study of underlying causes and designing therapeutic strategies. *Cre Expressing* strains that utilize nestin or Purkinje cell protein 2 promoters are useful for generating tissue-specific deletion mutants. *Neuronal Imaging* mutants express a variety of fluorescent proteins allowing visualization of various neuronal subsets. Since its inception, the IMR has accepted over 870 different induced mutant mouse strains and distributes over 100,000 mice annually to the scientific community. Current growth in the IMR collection is approximately 60 new strains each year. Each strain accepted into the IMR is cryopreserved (sperm or embryo) to protect against accidental loss and genetic contamination. An online resource allows researchers to retrieve information related to phenotype, strain construction, husbandry and relevant citations. Researchers wishing to submit mutant strains for consideration in the IMR may use the form available at the IMR web site. The IMR database can be accessed at URL: <http://www.jax.org/resources/documents/imr/>.

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PHR1 protein-protein interactions in retina and brain. *S. Xu, D. Valle.* Howard Hughes Medical Institute, Inst Genetic Medicine, Johns Hopkins Univ, Baltimore, MD.

PHR1 is a PH domain containing integral membrane protein predominantly expressed in primary sensory neurons, including photoreceptors, olfactory receptor neurons and the cochlear and vestibular hair cells (1). Using alternative promoters and alternative splicing, PHR1 encodes four isoforms: isoform 1 predominates in photoreceptors; isoform 4 in brain. Stimulated by this intriguing expression pattern, we produced a mouse lacking *Phr1* but found no obvious phenotype. We therefore turned to a yeast two-hybrid analysis, using PHR1 isoform 1 and isoform 4 as baits, to screen bovine retinal and human brain cDNA libraries, respectively. We found that PHR1 isoform 1 interacts specifically with itself and with carboxypeptidase E (CPE), while PHR1 isoform 4 interacts with the C-terminal, proline-rich region (PPr) of brain specific RGS9-2 (Regulator of G-protein Signaling 9-2). The latter agrees with the observation of Jones et al, who found PHR1 isoform 4 as an interactor with RGS9-2 bait (personal communication). To confirm these interactions, we co-expressed epitope-tagged versions of the interacting proteins in human HEK293 cells and used co-immunoprecipitation to detect interactions. We were able to confirm the self-interaction of the PHR1 isoform and the interactions of the appropriate PHR1 isoform with CPE and RGS9-2. Surprisingly, we also found interactions between PHR1 and its paralog, PHR2, raising a possibility that PHR1 and PHR2 form heterooligomers in certain cells. Our results define a system of *Phr1* interactors in neural cells and suggest additional studies to elucidate the function of PHR1. (1) Xu S, et al. PHR1 encodes an abundant, pleckstrin homology domain-containing integral membrane protein in the photoreceptor outer segments. *JBC* 1999 Dec 10;274(50):35676-85.

Detection of a large number of specific mRNA species in very few glial cells. *G. Yue¹, H. Wang¹, M. Luo¹, D. Frikker¹, K. Yin², J. Neubauer³, H. Li¹.* 1) Mol Gen & Microbio, CINJ-UMDNJ-RWJMS, New Brunswick, NJ; 2) Dept Cell Bio, UMDNJ-SOM, Stratford, NJ; 3) Dept Med, UMDNJ-RWJMS, New Brunswick, NJ.

Examining gene expression globally in single or very few cells in CNS is highly desired. The present study was designed to detect a panel of 58 mRNA species with a two-round RT-PCR/PCR protocol in few cells. 58 mRNA sequences relevant to hypothalamus function in the public database were selected for this study. Primers were designed for multiplex RT-PCR of the 58 mRNAs. Each primer pair was designed to flank a large intron sequence in the corresponding gene. If no intron information was available in the database for the rat, positions were based on available information of highly related genes in other evolutionarily closely related species. In this way, amplified products from mRNAs and from genomic DNA can be discriminated. Eight cells from a tumor cell line, C6 derived from rat brain glial cells, were used as starting material. RT-PCR was performed in the presence of the 58 primer pairs. Aliquots from the RT-PCR products were re-amplified separately with single primer pairs for each mRNA. In control reactions, each primer pair was used to amplify their target sequences in rat genomic DNA without reverse transcription. Single bands were detected with 19 (33%) pairs of primers while no comparable bands were detected in the controls indicating that these pairs of primers may have had their sequences separated by large introns in the rat genome. Amplification was detected in the RT-PCR products and the respective controls for 12 (21%) pairs of primers indicating that these primers may have had their sequences located in the same exons. No visible bands were detected from the amplification of both RT-PCR and respective controls with 21 (36%) primer pairs. It is likely that the sequences of these pairs flank large introns and these genes were not expressed at a detectable level or not expressed. Our results show that it is possible to analyze a large number of specific mRNA species in very few cells. The approach could be very useful for understanding the molecular mechanisms underlying the function of very few specialized cells in CNS.

Direct interaction between the Breast Cancer Tumor Suppressor Protein, BRCA1, and the Fanconi Anemia protein, FANCA. *A. Folias¹, M. Matkovic¹, D. Bruun¹, S. Reid¹, J. Hejna¹, M. Grompe^{1,2}, A. D'Andrea³, R. Moses¹.* 1) Dept. of Molecular and Medical Genetics, Oregon Health & Science University, Portland, OR; 2) Dept. of Pediatrics, Oregon Health & Science University, Portland, OR; 3) Dept. of Pediatric Oncology, Dana-Farber Cancer Institute and Dept. of Pediatrics, Childrens Hospital, Harvard Medical School, Boston, MA.

Fanconi anemia (FA) is a rare autosomal recessive disease characterized by skeletal defects, anemia, chromosomal instability and increased risk of leukemia. At the cellular level FA is characterized by increased sensitivity to agents that form interstrand crosslinks(ICL) in DNA. Six FA genes have been cloned and interactions among individual FANC proteins have been found. The FANCD2 protein co-localizes in nuclear foci with the BRCA1 protein following DNA damage and during S-phase, requiring the FANC A, C, E, and G proteins to do so. This finding may implicate the BRCA1 protein in DNA interstrand crosslink repair. Therefore, interactions between BRCA1 and the FANC proteins were investigated. Among the known FANC proteins, we find evidence for direct interaction only between FANCA and BRCA1. The evidence rests on three different techniques: yeast two-hybrid analysis, coimmunoprecipitation from in vitro synthesis, and coimmunoprecipitation from cell extracts. The interaction does not depend on DNA damage, thus FANCA and BRCA1 are constitutively interacting. The demonstrated interaction directly connects BRCA1 to the FA pathway of DNA ICL repair.

Tuberin Regulates Rho Activation, Cell Adhesion, and Cell Migration. A. Astrinidis¹, T. Cash¹, D. Hunter², C. Walker², J. Chernoff¹, E.P. Henske¹. 1) Dept. Medical Oncology, Fox Chase Cancer Ctr, Philadelphia, PA; 2) MD Anderson Cancer Ctr, University of Texas, Smithville, TX.

Tuberous sclerosis complex is a tumor suppressor gene syndrome characterized by benign tumors, seizures and mental retardation. Tuberin, the *TSC2* product, has a region of homology with Rap1GAP, and has been linked to cell cycle regulation, endocytosis, and steroid hormone function. Hamartin, the *TSC1* product, interacts with the ezrin-radixin-moesin cytoskeletal proteins, activates Rho, and promotes stress fiber formation. Hamartin and tuberin interact in vivo. Here we report that tuberin regulates cell adhesion, cell migration, and activation of Rho.

ELT3 cells, lacking endogenous tuberin, and MDCK cells, were transduced with a retroviral construct containing human *TSC2* cDNA. Tuberin expressing stable clones were selected in G418. Stable vector clones were used as controls in subsequent experiments. Tuberin-overexpressing ELT3 and MDCK cells exhibited increased attachment by 1.5-2 fold. The chemotactic migration of tuberin-expressing ELT3 cells was reduced by 60%. To define the biochemical pathways linking cell adhesion and migration with tuberin overexpression, Rho activation was assayed by Rhotekin pulldown. A 2-3 fold activation of Rho was present in MDCK and ELT3 cells overexpressing tuberin. Focal Adhesion Kinase (FAK), which is a downstream target of Rho, had increased phosphorylation by 2-4 fold in tuberin-overexpressing MDCK cells, and the total amount of FAK was decreased.

In conclusion, we demonstrate, for the first time, that tuberin expression increases cell adhesiveness and decreases cell migration. These changes are associated with increase in GTP-Rho and the phosphorylated fraction of FAK. These findings may have relevance to the pathogenesis of pulmonary LAM in patients with *TSC2* mutations.

Cloning and characterization of two novel human transcripts at the 9p12-p13 chromosomal region. *I. Eisenberg¹, M. Barash¹, H. Hochner¹, T. Kahan², T. Levi³, S. Mitrani-Rosenbaum¹.* 1) Molecular Biology, Hadassah Hospital, The Hebrew University-Hadassah Medical School, Jerusalem, Israel; 2) Bioinformatics Unit, The Hebrew University-Hadassah Medical School, Jerusalem, Israel; 3) Department of Genetics, Harvard Medical School, Boston, USA.

As part of the positional cloning strategy towards the successful identification of the gene causing Hereditary Inclusion Body Myopathy (HIBM, OMIM 600737) we have previously established a high throughput physical and transcriptional map spanning about 1Mb at the 9p12-p13 chromosomal region. Many studies have shown that multiple regions across the short arm of human chromosome 9 and in particular the region 9p12-p13 are functionally involved in a large number of common diseases such as lung cancer, hepatocellular carcinoma, papillary renal cell carcinoma, squamous cell head and neck cancer, bladder transitional cell carcinomas, melanoma, acute lymphoblastic leukemia, prostate cancer as well as in several rare diseases. Among the 25 distinct transcripts we mapped, 8 were previously characterized genes and 17 were ESTs of unknown function. Further analysis of two of these transcripts led to the identification and characterization of two novel genes. The first, C9orf19, encodes a conserved putative protein of 154 amino acids with an SCP-like extracellular protein domain. Its mRNA consists of 1.9kb widely expressed in adult tissues, most abundantly in lung and leukocytes. To gain insight into the biological function of C9orf19 gene, the putative protein was further analyzed for homologies and functional motifs. The second gene identified, RNF38 (RING finger protein 38), is a new member of the RING finger protein family. The complete mRNA consists of 6.8kb widely expressed in human tissues as a single transcript, most abundantly in testis. The predicted proline-rich protein consists of 432 amino acid residues with a coiled-coil motif, KIL motif and a RING-H2 motif at its carboxy-terminus. Further studies will determine the functional significance and biological role of these genes, which could be involved in one of the numerous human malignancies and other various diseases located in the p12-p13 segment of chromosome 9.

Understanding the Endothelial Function of Utrophin. *J.T. Ehmsen, A. Weir, K.E. Davies.* Functional Genetics Unit, Department of Human Anatomy and Genetics, University of Oxford, Oxford, England.

The critical role of the membrane-associated dystrophin-glycoprotein complex (DGC) in muscle is underscored by the large number of muscular dystrophies associated with mutations in its distinct protein components. Clinical and molecular correlations have helped identify the canonical members of the DGC in striated muscle, including dystrophin, the dystroglycans, sarcoglycans, dystrobrevins, syntrophins and sarcospan, and implicate a dual role in signaling and structural maintenance. Dystrophin-dystroglycan complexes are distributed in a number of nonmuscle cells as well, showing small variations on a theme in terms of associated proteins or isoform identities. Undertaking a survey of tissue-specific expression patterns of the two known utrophin isoforms, we have recently found that utrophin B is present exclusively in capillary endothelia. Vascular endothelia experience constant changes in hemodynamic stress and likely demand suitable protection against injury from repeated cycles of contraction and relaxation, yet must remain flexible enough to sustain a dynamic response. A utrophin-associated protein complex could participate in achieving this compromise. We are searching for the presence of other known dystrophin-associated proteins in endothelia using Western analysis, immunofluorescence, coimmunoprecipitation and yeast two-hybrid analyses to characterize specific interactions and to define potentially unknown binding partners of utrophin B. Dystrobrevin, syntrophin, and e-sarcoglycan have already been detected in endothelial cells, forming a group of candidate binding partners for endothelial utrophin B in at least some tissues. A closer examination of any subtle vascular defects in utrophin-deficient mice may also enhance our understanding of utrophin's endothelial function.

Mutations in the HERG ion channel: A novel link between long QT syndrome and sudden infant death syndrome. *M. Christiansen¹, N. Tonder², L.A. Larsen³, P.S. Andersen¹, H. Simonsen¹, N. Oyen⁴, J.K. Kanter⁵, J.R. Jacobsen⁶, I. Fosdal⁷, G. Wettrell⁸, K. Kjeldsen⁹.* 1) Dept Clinical Biochemistry, Statens Serum Inst, Copenhagen, Denmark; 2) Dept Internal Medicine, Hillerd Hospital, Hillerd, Denmark; 3) The Wilhelm Johanssen Centre for Functional Genome Research, Dept of Medical Genetics, Univ Copenhagen, Copenhagen, Denmark; 4) Dept of Clinical Genetics, Haukeland Hospital, Bergen, Norway; 5) Dept of Cardiology P, Gentofte University Hospital, Gentofte, Denmark; 6) Dept of Pediatrics, Rigshospitalet, Copenhagen, Denmark; 7) Dept of Pediatrics, Visby Hospital, Visby, Sweden; 8) Dept of Pediatrics, University of Lund, Lund, Sweden; 9) Medical Dept B, The Heart Centre, Rigshospitalet, Copenhagen, Denmark.

Sudden infant death syndrome (SIDS) remains - despite a dramatic decrease in prevalence during the last 15 years caused by the introduction of supine sleeping - the most important cause of death in children below 1 year of age. Prolonged cardiac repolarization, i.e. increased QT-time on the electrocardiogram, is a risk factor for SIDS. This has raised the interest for the role of congenital long QT syndrome in babies dying from SIDS. In a SIDS baby we identified a mutation, K101E, in the gene *KCNH2*, encoding the HERG potassium channel alpha-subunit. The mutation was - later - found associated with prolonged QT-time and the presence of torsade de pointes arrhythmia - a characteristic of long QT syndrome - in family members. Reduced HERG function, as may be seen in hypokalemia, drug-induced or sympathetic stimulation may be a common mechanism of arrhythmic death in children. A SIDS diagnosis should only be made after a careful genetic examination for mutations in LQTS associated genes. A screening of DNA from 120 SIDS patients for the mutation did not find more cases. Likewise no pore-associated mutations were found. Thus, classical Romano-Ward syndrome does not seem to play a major role in SIDS.

Identification of a Novel Alternatively Spliced isoform of human Fibulin-2 Gene Abundantly Expressed in Heart and Genetic Evaluation in Patients with ARVD. *D. Li, A.J. Marian, R. Roberts.* Dept Medicine/Cardiology, Baylor Col Medicine, Houston, TX.

We recently mapped a locus responsible for Arrhythmogenic Right Ventricular Dysplasia (ARVD) to 3p23. The disease is associated with apoptosis and fibrosis in the right ventricle and high incidence of arrhythmia and sudden death and is inherited as autosomal dominant. One of the genes mapped within the 8 cM region is fibulin 2. Fibulin-2 belongs to an emerging family of extracellular matrix proteins with a unique amino terminal extension as compared to other fibulins. Fibulin-2 is ubiquitously expressed in human tissues including the heart. To detect mutations such as those in splice junctions, it was necessary to determine the genomic structure of the gene. Analysis of the cDNA from human heart and other tissues by RT-PCR, showed two distinct bands differing by 140 base pairs. The sequence of the smaller band, identical to the previously reported cDNA, had ubiquitous expression in human tissues. The larger band with additional 140 base pairs had restricted expression with abundance in the heart, moderate in skeletal muscle and pancreas and a trace in the liver. Comparison of fibulin-2 cDNA sequence, with its genomic counterpart sequence, identified 17 exons. The larger band had an additional exon which in the genomic sequence was exon 9. Exon 9 had been spliced out of the smaller form. The predicted amino acid sequence encoded by exon 9 of human fibulin-2 is highly conserved across species with 95% identity to that of mouse fibulin-2. A complete exon-intron organization was derived and all exons and their flanking introns amplified and sequenced from patients DNA. Several polymorphisms were detected, but no disease-causing mutation responsible for ARVD was identified. Thus, there are two alternatively spliced transcripts of the fibulin-2 gene, one with 4,139 bp ubiquitously expressed in human tissues and another with 4,280 bp with restricted tissue expression but abundantly expressed in the heart. While the function of fibulin-2 remains to be defined, the distinct tissue expression of the two forms should accelerate further clinical and functional investigation.

Fang-1, Which Can Increase Expression of IRS1, is a Candidate Gene for Diabetes Mellitus. *Y. Chang, J. Zuo, Y. Li, F. Fang.* Department of Molecular Biology and Biochemistry, Institute of Basic Medical Science, Peking Union Medical College & Chinese Academy of Medical Sciences, 5 Dong Dan San Tiao, Beijing, P.R.China.

A novel gene, which is related to blood-glucose regulation and is named as Fang-1, was cloned by messenger RNA differential display. First, the blood glucose regulation model of rat was build with jugular vein right atrium intubations. One differentially expressed fragment was proved by slot blot and Northern blot analysis, and then full-length cDNA was cloned by screening the muscle cDNA library of rat, which has homologue in human. After transfecting Fang-1 into L-6TG cells, we found Fang-1 was localized in cytoplasm and cell nucleus. Overexpression of Fang-1 in CBRH7919 can up-regulate the expression of IRS1, the key factor related to glucose metabolism. Since Fang-1 itself was up-regulated after glucose stimulation *in vivo*, and it can also up-regulate the expression of IRS1, we propose that Fang-1 might be a novel candidate gene for diabetes mellitus and helpful to clarify the mechanism of diabetes mellitus.

Proteolytic inactivation of DNA ligase III during apoptosis converts the base excision DNA repair pathway into a cell-killing machine. *L. Bordone*², *C. Campbell*¹. 1) University of Minnesota, Dept. Pharmacology, Medical School, 321 Church St. SE Minneapolis, MN 55455; 2) MIT, Department of Biology, Cambridge MA.

Exposure of cells to anti-Fas monoclonal antibody (Fas) results in the proteolytic cleavage of DNA ligase III (Lig3). Lig3 participates in the base excision repair (BER) pathway, sealing nicks produced during the repair reaction. Loss of Lig3 would lead to the accumulation of chromosome single-strand breaks, which we hypothesized could contribute to the cell death induced by Fas. We further proposed that this DNA damage could contribute to the cell death observed following exposure of cells to Fas. We performed a series of experiments to test this hypothesis. We created cells that expressed protease-sensitive or protease-resistant alleles of Lig3 and found that while 73% of cells overexpressing a protease-sensitive allele died following exposure to Fas, cells overexpressing the protease-resistant allele were completely protected. We also used pharmacologic and genetic approaches to determine if blocking the BER pathway upstream of the introduction of nicks into the DNA phosphodiester backbone would protect cells from Fas-induced death. We found that 90% of cells pretreated with methoxyamine (MX), an inhibitor of APE1, the enzyme that introduces nicks into the DNA phosphodiester backbone during BER, survived treatment with Fas. In contrast, only 72% of cells that were not treated with MX prior to Fas exposure survived. We also found that 90% of cells transfected with antisense APE1 survived treatment with Fas, compared to 75% of control cells. These data are consistent with the hypothesis that Fas-mediated degradation of Lig3 converts the BER pathway from a cell protector that repairs DNA damage, to a cell-killer that generates DNA damage. We propose that the DNA single-strand breaks that accumulate in cells in which Lig3 has been degraded contribute to the death that follows exposure of cells to Fas. We have generated additional data indicating that inhibition of BER in this manner also occurs in cells subjected to serum starvation, suggesting that this may represent a common mechanism through which cells ensure efficient death in response to a variety of apoptotic stimuli.

Genomic Organization and Characterization of the Human ATX1 Homologue Involved in Copper Homeostasis.

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Copper is an essential trace element that plays a critical role in the biochemistry of iron homeostasis, cellular respiration, antioxidant defense, neurotransmitter biosynthesis, connective tissue biosynthesis and pigment formation and is needed for the survival of all living organisms. Menkes disease and Wilson disease are inherited disorders of copper transport caused by mutations in copper ATPase genes *ATP7A* and *ATP7B*, respectively. Molecular genetic studies of these disorders have provided opportunities to better understand the mechanisms of cellular copper metabolism. In addition, studies in yeast have revealed a family of cytoplasmic proteins termed copper chaperones that bind copper ions and deliver them to specific cellular pathways. Biochemical studies of the copper chaperone *Atox1* showed interaction between this protein and the Menkes and Wilson disease copper transporters. While no disease-associated mutations have been reported in the human homolog of *Atox1*, mice with disruption of the murine homolog demonstrated perinatal mortality similar to that observed in the *brindled* mice (*Mo^{br}*), a mouse model of classical Menkes disease. In this study, we characterized the genomic structure of the *Atox1* human homolog. We identified 4 exons within a genomic distance of approximately 14 kb. The *Atox1* start codon is located in the 3' end of exon 1 and the termination codon is in exon 3. Characterization of the genomic structure of the *Atox1* human homolog will facilitate screening of this gene using genomic DNA, particularly appropriate in patients with Menkes or Wilson disease phenotypes who do not have molecular alterations in *ATP7A* or *ATP7B*.

Genetic basis for non-syndromic and syndromic, Blepharophimosis/Ptosis and Epicantus Inversus Syndrome

(BPES)-associated Premature Ovarian Failure (POF). *L. Crisponi¹, M. Uda^{1,9}, F. Chiappe², M. Deiana¹, G.L. Usala¹, M. Marongiu¹, P. Amati³, D. Bonneau³, F. Faravelli⁴, J. Tolmie⁵, L. Bisceglia⁶, M.R. Piemontese⁶, L. Zelante⁶, P. Gasparini⁶, G. Crisponi², L. Boccone², A. Iolascon⁷, A. Percesepe⁸, A. Forabosco⁸, G. Pilia¹ and FOXL2 study group.* 1) Inst Ric Talass Anemie Medit, Cons Nazion delle Ricerche, Cagliari, Italy; 2) University of Cagliari, Cagliari, Italy; 3) Service de Genetique Medicale, Poitiers, France; 4) Ospedale Galliera, Genova, Italy; 5) Duncan Guthrie Ins. Med. Genet., Glasgow, UK; 6) IRCCS, San Giovanni Rotondo, Italy; 7) University of Bari, Italy; 8) University of Modena, Italy,; 9) NIA, NIH, USA.

In Type I BPES (MIM110100) eyelid abnormalities are associated with Ovarian Failure leading to Female Infertility. We have recently cloned a winged helix/forkhead transcription factor gene, FOXL2, mutated in BPES. We report here a FOXL2 mutation screening on 43 BPES and 71 POF patients. Nonsense and frameshift mutations were identified on all 13 type I BPES families analyzed. Milder mutations were found in 9 out of 13 type II BPES patients with a typical hypomorphyc phenotype. We detected 9 FOXL2 mutations in 17 patients whose clinical type could not be determined. The 12 families with no apparent mutation could harbor either lesions outside the FOXL2 gene or mutations in another gene. In two such families linkage analysis excluded the involvement of the region containing FOXL2 in the pathogenesis. Mutations in the TWIST gene, involved in Saethre-Chotzen Syndrome (MIM101400) in which craniosynostosis is often associated with eyelid anomalies, have not been detected in any of the FOXL2 negative families. A possible role of FOXL2 in non-syndromic Female Infertility has been tested in 71 females with idiopathic POF. We analyzed the entire coding region, proximal promoter, 5UTR, and 3UTR. We have identified a missense, a promoter, a 5UTR and 3 different 3UTR mutations. The role of these mutations in the pathogenesis of this common disorder is currently under investigation with two complementary approaches: genetic (association studies) and functional analysis on the gene and its product.

Toward Temporal and Spatial Control of Retinal Gene Expression. *S.H. Tsang¹, M.L. Woodruff¹, M. Jiang¹, G. Fain¹, C.S. Lin², S.P. Goff², D.B. Farber¹.* 1) Jules Stein Eye Inst, UCLA Sch of Med, Los Angeles, CA; 2) Columbia U., New York, NY.

Purpose: The *Pdeg^{tm1}/Pdeg^{tm1}*, mutant mouse (carrying a targeted deletion of the gene encoding PDEg, *Pdeg*) lacks PDE activity and undergoes rapid retinal degeneration. An interaction between the inhibitory PDEg and catalytic PDEab subunits may be critical for the proper folding, conformation or stabilization of the PDE enzyme complex.

Unfortunately, the maldeveloped and truncated outer segments of the *Pdeg^{tm1}/Pdeg^{tm1}* mouse prohibit their use in biochemical studies. A fully developed photoreceptor that lacks PDEg will allow us to test if PDEg has a positive role in the formation of an active PDE complex. We will develop an inducible gene expression system that will make possible to delete or activate genes in an adult mouse. **Methods:** Fluorescence microscopy was used to monitor floxed-Pdeg--IRES-EGFP expression in the retina of mice carrying a modified allele of the PDEg gene (*Pdeg*). EGFP expression in single rods was detected in the suction electrode after stimulation at 488 nm. **Results:** Transgenic mouse lines were established using embryonic stem cells carrying a Pdeg floxed allele with an IRES-EGFP cassette introduced into its 3' end. To test the expression of this construct in 293T cells, in vitro, a CMV promoter was placed at its 5' end. EGFP fluorescence was detected after transfection. The floxed-Pdeg-IRES-EGFP cassette (without the CMV promoter) was then introduced into the mouse germline. Sections of retinas from the resulting mice showed that EGFP was specifically expressed in photoreceptor cells. In vivo, Cre-mediated site-specific recombination should result in loss of both *Pdeg* and EGFP expression. **Conclusion:** We have successfully used EGFP as a reporter for the expression of PDEg. The lines of floxed mice that we have generated will be utilized to monitor Cre-mediated recombination in photoreceptors. An inducible gene targeting system may allow us to address several previously unapproachable problems in sensory biology.

Growth hormone expression on mRNA level is significantly reduced in the brain of *Ndp*-knockout-mice. U.

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Norrie disease (ND, OMIM # 31060) is a rare X-linked recessive neurological syndrome characterized by congenital blindness, progressive deafness and mental retardation. It is caused by mutations in the *Ndp* gene (Norrie disease, pseudoglioma). Mutations in this genes were also observed in patients with exudative vitreoretinopathy, Coats disease and retinopathy of prematurity. The *Ndp* gene in mouse is expressed in the retina, the chochlea, the olfactory bulb and the Purkinje cell layer of the cerebellum. *Ndp* codes for a small extracellular protein of unknown function containing a cystine knot motif characteristic for several growth factors, including transforming growth factor beta. The expression pattern of *Ndp* in the eye and the ear corresponds to the progressive and degenerative morphological changes in these organs in the knockout mice. So far, no morphological alterations were described in the brain of knockout animals. To search for transcriptional changes in the brain of knockout mice we performed gene expression profiling by cDNA-microarrays. By comparative hybridisation of total brain RNA from one year old wildtype and knockout mice to a brain specific cDNA-microarray we identified several differentially expressed genes and confirmed differential expression of the Growth hormone (*mGH*) mRNA on Northern Blots and by RT-PCR. Using RNA from wildtype and *Ndp* knockout mice of different ages (P14, P21, P28, 3 month, 6 month and 1 year) we now show that the growth hormone mRNA expression is increased at P14 in *Ndp* knockout mice compared to wildtype, while it decreases from P21 onwards. Therefore, there seems to be a switch in GH expression in the brain between P14 and P21, the time when *Ndp* expression is enhanced. These findings suggest that there might be a relationship between the partially overlapping *Ndp* and the *mGH* expression in the brain. The *Ndp* mouse model may help to characterize this tissue-specific effect.

Analysis of histone H1 subtype associations with the Barr body. *W.H. Brooks¹, B. Hong¹, M.H. Parseghian⁵, B.A. Hamkalo⁴, T.P. Yang^{1,2,3}.* 1) Biochem & Molec Biology, Univ Florida, Gainesville, FL; 2) Center for Mammalian Genetics, Univ Florida, Gainesville, FL; 3) Division of Pediatric Genetics, Univ Florida, Gainesville, FL; 4) Molec Biology & Biochem, Univ California, Irvine, CA; 5) Peregrine Pharmaceuticals, Tustin, CA.

One of the two X chromosomes in each female mammalian somatic cell is transcriptionally inactivated to achieve dosage compensation between male and female cells for most X-linked genes. The resulting inactive X chromosome forms the late-replicating heterochromatic Barr body seen in female somatic cell nuclei at interphase. The molecular composition and structure of the Barr body are largely unknown with few molecular components currently identified. The linker histone H1, consisting of at least 8 subtypes in mammals, has been associated with the formation of higher order chromatin structure, chromatin condensation, and transcriptional silencing. Each of the H1 subtypes is encoded by a different gene and all show different expression patterns and cell type distributions. Using affinity-purified polyclonal subtype-specific antibodies and detection by indirect immunofluorescence, we have examined the association of three H1 subtypes with the Barr body. We find subtypes H^S-1, H^S-2, and H^S-3 are preferentially localized to the Barr body in human female fibroblasts. The accumulation of these histone H1 subtypes in the Barr body, along with the reported depletion of subtype H^S-2 in active chromatin, suggests a role for histone H1 in the structure, maintenance, and/or function of the Barr body in female nuclei.

Evolutionary analysis of a cluster of ATP-binding cassette (ABC) genes reveals multiple gene duplication, gene deletion and gene conversion events. *T. Annilo*¹, *Z.-Q. Chen*², *S. Shulenin*¹, *M. Dean*¹. 1) Human Genetics Section, Laboratory of Genomic Diversity, NCI-Frederick, MD 21702; 2) Intramural Research and Support Program, SAIC-Frederick, MD 21702.

The proteins of the ATP-binding cassette (ABC) superfamily utilize the energy of ATP hydrolysis to transport a wide variety of molecules across different cellular membranes. In humans, current annotation classifies 48 ABC proteins into seven subfamilies. While most of the ABC genes are dispersed in the mammalian genome, five ABCA subfamily genes (*ABCA5*, *ABCA6* and *ABCA8-10*) form a unique cluster on human chromosome 17q24. To investigate the evolutionary history of ABC transporters, we characterized the structure and expression pattern of the genes at the mouse conserved syntenic locus. Phylogenetic and comparative sequence analysis reveals that after the divergence of rodent and primate lineages, at least one gene has been lost in each genome. In addition, we found that both mouse and human clusters show evidence of a number of gene conversions, in several cases involving intron sequences. Analysis of exon-intron structures indicates that splice sites are more conserved than exon sizes and only those nucleotide insertions and deletions that do not disrupt the open reading frame within the established splicing pattern have been fixed in the course of evolution. Similarity of both the genomic organization and primary structure among the genes in this cluster suggests that the duplications generating the cluster occurred relatively recently compared to most of the ABC genes in the present-day mammalian genomes. For instance, the *Fugu rubripes* genome contains an ortholog of *ABCA5*, but not of any other member of the ABCA cluster. This also supports the hypothesis that *ABCA5* has been a founder gene of that cluster.

Expression of the gene for progressive ankylosis (ANK) during insulin-induced chondrogenesis in ATDC5 cells.

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ANK codes for a transmembrane protein that appears to regulate intra- and extracellular levels of inorganic pyrophosphate. Missense mutations in ANKH, the human homologue of ANK, have been reported by us and others in the autosomal dominant arthropathy known as calcium pyrophosphate dihydrate deposition disease. To ultimately understand the impact of mutations in ANK on its function in chondrocytes and articular cartilage, we have chosen to use the ATDC5 cell line as a chondrogenic model system. ATDC5 is a clonal murine cell line established from a differentiated culture of embryonic cell-derived AT805 cells. When cultured in the presence of insulin, the cells undergo a program of chondrogenesis that includes the induction of chondrocyte-specific gene expression. Cells were plated at low density and grown as monolayers with and without the addition of insulin for a period of 28 days. Media were changed every other day and cells were harvested on days 1, 3, 7, 14, 21, and 28 for histochemical analyses and RNA isolation. Consistent with previous reports, we observed weak alcian blue staining of isolated clusters of cells by day 7 of culture of cells treated with insulin. By day 14, the alcian blue staining was strong in areas of densely packed multicellular nodules. Analyses of transcripts for cartilage-expressed genes by relative quantitative RT-PCR were consistent with the induction of chondrogenesis; for example, the expression of pre-chondrogenic collagen IIA was strong at early time points, whereas that of chondrocyte-specific collagen IIB predominated at day 14 and later time points. ANK transcript was present from day 1 of culture and was similar in cells with or without treatment with insulin. Since TGF β has been reported to significantly increase the production of extracellular inorganic pyrophosphate in chondrocytes, we also cultured the ATDC5 cells in the presence of insulin plus TGF β . We did not observe a significant increase in ANK expression in TGF β -treated cells compared to those treated with insulin only. These studies provide the foundation for future analyses of mutated ANK in the ATDC5 line. (Supported by AR44360 from NIH/NIAMS to C.J.W.).

Specific expression of Cre recombinase in kidney tubular cells of mice. *L. Tontsidou*¹, *K. Bilbilis*¹, *P. Pennekamp*¹, *B. Skryabin*², *H-J. Galla*³, *J. Horst*¹, *B. Dworniczak*¹. 1) Institut of Human Genetics, Institute of Human Genetics, UKM Muenster, Germany; 2) ZMBE Muenster, Institute of Experimental Pathology, Germany; 3) Institut fuer Biochemie, Muenster, Germany.

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common monogenic diseases in humans with an incidence of 1:600. The cardinal finding is the formation of fluid filled cysts in both kidneys - predominantly in the proximal tubules - frequently resulting in end stage renal failure. In the vast majority of cases, the disease is caused by mutations in PKD1 and/or PKD2. However, despite extensive research only little is known concerning the pathogenesis of ADPKD. Cystogenesis in ADPKD kidneys in humans is a focal process: patients inherit one germline mutation and acquire somatic mutations affecting the normal allele in a subset of cells (second hit). In the last couple of years conventional knockout mouse models for Pkd1 and Pkd2 have been generated. All of them are prenatal lethal making it impossible to analyze the pathogenesis of ADPKD after birth/over a longer period in adult mice. These limitations caused by embryonic lethality can be circumvented by conditional mutagenesis in which the loss of gene function is placed under temporal and/or spatial control. In order to establish this system we have generated a transgenic mouse line expressing the Cre-recombinase under the control of a truncated mouse type II GGT promoter. We expected that this promoter allows the spatial excision of loxP flanked genes in proximal tubular cells. The recombinase activity was demonstrated by crossing these mice with Cre- reporter lines (Z/AP and ROSA26). Both lines carry a marker gene whose expression requires excision of loxP flanked stop sequences. Using alkaline phosphatase histochemistry in Z/AP mice as well as b-Galactosidase histochemistry in ROSA26 mice we observed a strong and specific expression of Cre-recombinase in proximal tubular renal cells. This Cre transgenic mouse line, which induces efficient Cre-mediated excision of DNA in proximal tubules, should provide a useful genetic resource to elucidate the role of loxP manipulated genetic targets in cyst formation of ADPKD.

A mutation in FSHb as a possible cause of idiopathic hypogonadotropic hypogonadism. A.D. Clark^{1,2}, R.J. Sherins³, D.P Bick⁴, L.C. Layman^{1,2}. 1) Section of Reproductive Endocrinology, Infertility, & Genetics, Department of Obstetrics and Gynecology, Medical College of Georgia, Augusta, Georgia; 2) Neurobiology Program, Institute of Molecular Medicine & Genetics, Medical College Georgia, Augusta, GA; 3) Genetics and IVF Institute, Fairfax, Virginia; 4) Department of Genetics, University of Wisconsin.

Follicle stimulating hormone (FSH) is a pituitary protein consisting of a a and b subunit that is an important regulator of reproductive function in males and females. It is necessary for follicle maturation in females, spermatogenesis in males, and the aromatization of testosterone to estradiol. To date, four mutations in FSHb have been described which all resulted in varying degrees of pubertal development and low to undetectable FSH accompanied by elevated serum luteinizing hormone (LH). Idiopathic hypogonadotropic hypogonadism (IHH) is characterized by irreversible pubertal delay in patients with low serum levels of FSH and LH, and the absence of other endocrinologic abnormalities. It is conceivable that an inactivating mutation in FSHb could mimic the IHH phenotype of hypogonadism and delayed puberty. To investigate this hypothesis, the entire coding region of FSHb was analyzed by denaturing gradient gel electrophoresis (DGGE) in 76 patients with IHH and 20 controls for mutations in FSHb. Two unrelated IHH patients shared a sequence difference in FSHb exon two that was absent in all other patients and controls tested. Subsequent DNA sequencing showed both were heterozygous for a G®T substitution that results in an amino acid change of Ser1Ile. This sequence difference was introduced into a FSH expression vector and its bio- and immunoactivity was assayed *in vitro*. The Ser1Ile showed both decreased bio- and immunoactivity of FSH, indicating that it is a true mutation affecting FSH production *in vitro*. Our findings suggest that this mutation may be partly or completely responsible for the IHH phenotype in the individuals identified with this sequence difference. It is possible that the Ser1Ile might only be significant in the homozygous state, but we cannot exclude a dominant negative effect or modifying effects from other genes.

HNF-4 Alpha Enhances Expression of Human Glycerol Kinase in a Human Hepatocellular Carcinoma Cell Line.

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Isolated glycerol kinase deficiency (GKD) is an X-linked inborn error of metabolism for which genotype does not correlate with phenotype. We hypothesize that phenotypic variability might be due to factors influencing GK expression. We have shown previously that an HNF-4a (HNF-4) consensus binding site exists between -433 and -451 bp (GK-HNF-4 site) and that this region is important for enhanced GK expression. The purpose of this study was to investigate the specificity and functionality of HNF-4 binding to the GK-HNF-4 site. To determine the specificity of HNF-4 binding to the GK-HNF-4 site, rat liver nuclear extract was used in a gel mobility shift assay with a radiolabeled GK-HNF-4 site and excess amounts of unlabeled HNF-4 consensus oligonucleotide. One band was effectively competed by excess unlabeled HNF-4 consensus oligonucleotide. Repetition using an unlabeled mutated oligonucleotide (mGKHNF-4), with a three base pair change in the GK-HNF-4 site, did not compete. Self-competition demonstrated specificity of the interaction. An HNF-4 antibody showed a supershift of the HNF-4-GK complex, indicating that HNF-4 binds to the GK-HNF-4 site. To explore the functionality of HNF-4 binding to the GK promoter, cotransfection of HepG2 cells with GK500LUC (-1 to -500 bp construct) or mutant GK500LUC construct (mGK500LUC) with an HNF-4 expression vector showed that HNF-4 increased expression of GK500LUC by 2 fold, while transfection of mGK500LUC led to diminished baseline luciferase activity. These data suggest that HNF-4 binds to the GK-HNF-4 site and enhances expression. Identification of the role of HNF-4 and other transcription factors important for GK expression will permit us to examine possible interindividual variations in their response elements in the GK 5' UTR. These investigations will allow us to test the hypothesis that influences on transcriptional activity may mediate phenotypic variability in isolated GKD. Since mutations in GK and HNF-4 are associated with type 2 diabetes mellitus, then the involvement of HNF-4 in GK expression is particularly intriguing.

A YY1 Binding Site Functions as a Downstream Promoter Element (DPE) to Direct Accurate Transcriptional Initiation of the Human LINE-1 Retrotransposon. *J.N. Athanikar, J.V. Moran.* Departments of Human Genetics and Internal Medicine, University of Michigan, Ann Arbor, MI.

Long Interspersed Nuclear Elements (LINEs or L1s) are a family of non-Long Terminal Repeat (non-LTR) retrotransposons that can move from one genomic location to another via reverse transcription of an RNA intermediate. They comprise nearly 17% of the human genome sequences and though the vast majority (>99.8%) are retrotransposition-defective, it is estimated that 60-100 remain retrotransposition-competent (RC-L1s). RC-L1 retrotransposition is ongoing, and deleterious insertions have resulted in a number of genetic disorders, including cancer. The initial step in L1 retrotransposition is the transcription of L1 RNA from an internal promoter located within its 5'-untranslated region (5'UTR). Earlier studies had identified a YY1 (Yin Yang 1) binding site near the 5' end of L1 (+13 - +20) as a key *cis*-acting sequence required for transcription from a heterologous reporter, but little else is known about how L1 transcription is regulated. Here, we have employed a genetic assay to detect L1 retrotransposition in cultured cells in conjunction with a luciferase reporter system to test whether the YY1 binding site also is required for L1 retrotransposition. Unexpectedly, mutations in this site neither affected the activation potential of the L1 5'UTR, nor affected the ability of L1 to retrotranspose. Instead, RNase protection analysis demonstrated that these mutations abolish proper transcriptional initiation at the +1 start site. Thus, our data suggest that the YY1 binding site acts as a downstream promoter element (DPE) to direct proper L1 transcriptional initiation, thereby enabling L1 to undergo exponential amplification in subsequent generations.

Functional characterization of possible candidate genes deleted in Wolf-Hirschhorn syndrome. *S.U. Endeke¹, S. Schlickum¹, C. Steglich², N. Pfarr³, F.O. Fackelmayer⁴, A. Winterpacht¹.* 1) Institute of Human Genetics, University Erlangen-Nuremberg, Erlangen, Germany; 2) Institute of Human Genetics, University of Hamburg, Hamburg, Germany; 3) Children's Hospital, University of Mainz, Mainz, Germany; 4) Heinrich-Pette-Institute, Hamburg, Germany.

The Wolf-Hirschhorn syndrome (WHS, MIM194190) is caused by deletions in chromosome region 4p16.3 and is thought to be a contiguous gene syndrome with a yet unknown number of genes contributing to the phenotype. Recently, we and others identified several candidate genes in (and flanking to) a previously defined 165 kb WHS critical region. We have now focused our attention on the neurological and neuromuscular features of this syndrome and started with a detailed functional characterization of three putative candidate genes for this aspect of the phenotype. The first gene, *LETM1*, flanks the WHSCR and is deleted in almost all WHS patients. *LETM1* shows significant homology to proteins from different species which exhibit a characteristic domain structure including a transmembrane domain, 1-2 EF-hand motifs and a novel SAF/SAP related domain. We could show that in contrast to a standard SAF/SAP domain the SAF/SAP related domain possesses no conventional SAR/MAR-binding properties. The function of the novel domain remains unknown and will be analyzed further. To determine the subcellular localization of human *LETM1* we transfected COS7 cells with EGFP-cDNA-fusion constructs. Our results demonstrate that the fusion protein is located in the mitochondria, indicating that *LETM1* plays a role in mitochondrial function. Since mitochondrial dysfunction has gained considerable interest as a potential cause of epileptic seizures and neuromuscular disorders, it is tempting to speculate that *LETM1* represents a suitable candidate for these clinical features characteristic for WHS patients with a full phenotype. In addition, two other novel putative genes from the WHSCR are currently investigated. The genes, which we designated *WHSC3* and *WHSC5*, show a strong expression in developing and adult brain. RNA-in situ hybridizations revealed a cell type specific expression pattern for *WHSC3* suggesting a role in the neurological phenotype of WHS.

Evolutionary Conservation of the Myotonic Dystrophy Type 2 (DM2) Repeat Tract. *C.L. Liquori, J.W. Day, L.P.W. Ranum.* Institute of Human Genetics, University of Minnesota, Minneapolis, MN.

Myotonic dystrophy (DM), the most common form of muscular dystrophy in adults, can be caused by mutations on either chromosome 19 (DM1) or 3 (DM2). We recently demonstrated that a CCTG expansion in intron 1 of the *zinc finger protein 9 (ZNF9)* gene causes DM2. The DM2 repeat contains the complex repeat motif (TG)_n (TCTG)_n (CCTG)_n. The CCTG portion of the repeat tract is interrupted on normal alleles and expands on affected alleles. The *ZNF9* cDNA is highly conserved throughout evolution, with homologs in mouse, chicken, and yeast. To gain insight into possible functions of the DM2 repeat, located in an intron, we looked for evolutionary conservation in chimpanzee, gorilla, mouse, and rat. PCR was performed on genomic DNA from chimpanzee and gorilla using primers within intron 1 flanking the repeat. The 1.3 Kb region was highly conserved (chimpanzee: 98%; gorilla: 97%) and included similar but not identical complex repeat motifs. Chimpanzee has a (TG)_n TCTG (CCTG)_n, with interruptions in the TG but not the CCTG tract. Gorilla has a more complex (TG)_n (CCTG)_n TCTG CCTG (TCTG)_n and (TCTG)_n (CCTG)_n TCTG CCTG TCTG, separated by a 38 nucleotide stretch with homology to an Alu repeat. We also compared the entire intron 1 sequence of mouse, rat, and human. Intron 1 in both mouse (5.1 Kb) and rat (5.8 Kb) is much smaller than in the human gene (>20 Kb) and is conserved throughout its length between mouse and rat (85%). The mouse has a complex repeat motif, (TG)₅ TCTG TG (TCTG)₂, similar to that found in human but without a CCTG tract, whereas the rat only contains the (TG)_n portion of the repeat tract. A small region of intron 1 downstream from the repeat tract is conserved with human (mouse: 51%; rat: 48%). The evolutionary conservation of the DM2 repeat motif suggests a biological function. Given that both TG and tetranucleotide (UCAY) repeats have been shown to be involved in regulating alternative splicing, the normal role of the DM2 repeat tract may be to regulate alternative splicing of *ZNF9*.

Characterization of functional elements in the neurofibromatosis (NF1) promoter. *D.I. Rodenhiser, M.X. Zou, D.T. Butcher, T.C. Groves, S.P. Yee.* Cancer Research Labs, London Regional Cancer Centre, and The University of Western Ontario, London, ON, Canada.

Neurofibromatosis is a familial cancer syndrome attributable to mutations in the NF1 gene. Although considerable insight has been gained on the function of the neurofibromin protein, surprisingly little information is available related to NF1 transcriptional regulation. Previous work from our lab has identified an unmethylated CpG-rich island containing the putative NF1 promoter which is flanked by methylated sequences. In the present study, we have begun identifying and functionally characterizing the NF1 regulatory region, as well as evaluating the molecular mechanisms that regulate NF1 transcription during normal cellular differentiation and the events leading to NF1-related neoplasia. Overlapping regions of the NF1 proximal promoter have been cloned using a series of DNA fragments created by PCR and characterized for use as luciferase reporter constructs. Luciferase reporter assays have identified a 425 bp region displaying activities up to 80 fold higher than baseline reporter levels. Gel mobility shift assays confirm binding of SP1 and CRE to their putative recognition sequences and provide the first evidence identifying functional sites likely involved in regulating NF1 transcription. Mutations at the CRE and SP1 binding sites immediately upstream of the transcription start site have dramatic effects that lead to a 70-90 percent decrease in reporter activity. Our assays have also revealed a putative repressor region within the NF1 promoter region corresponding to CCCTC rich sequences between the transcription and translation start sites. As well, in vitro methylation of the NF1 reporter constructs supports a model where aberrant NF1 promoter methylation could repress transcription in a manner similar to other tumour suppressor genes. This study is providing a clearer understanding of the functional sites within the NF1 regulatory region that are responsible for regulation of NF1 gene expression and the critical elements that may be targeted for inactivation. (This research funded by the US Army / Department of Defense Neurofibromatosis Research Program.).

Functional characterization of protein domains in NYX (nyctalopin on chromosome X). *C. Zeitz*^{1,2,3}, *S. Schweiger*², *H. Scherthan*², *S. Freier*², *W. Berger*^{1,2}. 1) Dept. Med. Molecular Genetics, University of Zurich, Schwerzenbach, Switzerland; 2) Max Plank Institute of Molecular Genetics, Berlin, Germany; 3) Free University, Berlin, Germany.

CSNB1, the complete type of X-linked congenital stationary night blindness, is a nonprogressive retinal disorder characterized by impaired night vision, myopia, nystagmus and reduced visual acuity. The disease is caused by mutations in the CSNB1 gene (NYX). The predicted protein contains an N-terminal signal peptide, a C-terminal glycosylphosphatidylinositol (GPI) membrane anchor and 11 LRRs, flanked by cysteine-rich LRRs. Two previously identified GPI-anchored LRR proteins (chaoptin and connectin) have a pivotal role in photoreceptor cell adhesion as well as axon guidance processes. We isolated the mouse orthologue, mNyx, and compared the amino acid sequence to the human protein. Amino acid sequence identity is much higher in the LRR-core (>90%) than in the signal sequence and GPI anchor (62% and 52%, respectively). Protein localization and the functional relevance of predicted domains (signal sequence and GPI-anchor) were studied by expression of V5-tagged human and mouse constructs in COS7 and HeLa cells by fluorescence immunochemistry. Different protocols were established to localize the protein. When cells were permeabilized and extracted, NYX is confined to the ER and Golgi apparatus, which was proven by colocalization experiments. Using live cell staining against V5 we observed NYX in a punctuate pattern on the surface of the cell. Similar results were obtained with expression constructs containing the mouse cDNA sequence. Deletion of the GPI anchor resulted in a time dependent release of NYX from the cell surface but part of it remained in the ER and Golgi apparatus. Constructs lacking the signal sequence showed a diffuse pattern of NYX in the cytosol as do constructs with neither signal sequence nor GPI anchor. Thus, our data provide evidence that NYX is transported and attached to the cell membrane in both species, human and mouse. Adhesion studies using mutant variants will reveal whether NYX plays indeed an important role for protein interaction and axon guidance processes. >>.

Functional analysis of single-minded 2 (SIM2) gene located on human chromosome 21q22.2:promoter and nuclear localization signal. *Y. Shimizu¹, A. Yamaki¹, J. Kudoh², S. Minoshima², N. Shimizu²*. 1) Department of Medical Genetics, Kyorin Univ Sch Health Sci, Tokyo, Japan; 2) Department of Molecular Biology, Keio Univ School of Medicine,.

Human SIM2 gene encodes a PAS family transcription factor and it has been postulated to be a candidate gene for the pathogenesis of mental retardation in Down syndrome. To understand the molecular mechanism of SIM2 gene expression, we have analyzed the promoter region using a series of deletion constructs and point mutants fused with luciferase reporter gene and gel retardation assay. We found that the 60bp-sequence between nt-1385 and -1325 upstream of the translation initiation site has a significant promoter activity and that c-myb like transcription factor appears to regulate the transcription of human SIM2 gene in T98G cells. Furthermore, we analyzed more downstream region and found that a CAAT cis-element located between nt-1015 and -1004 appears to be important for SIM2 gene expression. To determine the nuclear localization signal of SIM2 protein, we have generated fusion genes of full-length cDNA and deletion mutants of SIM2 with green fluorescent protein (EGFP). Transient expression analysis in HeLa cells revealed that SIM2 protein exists in nuclei without any stimuli. The bHLH-PAS1-PAS2 and HST domains are not necessary for the nuclear localization of SIM2 protein and the region of codon 367 to 389 are sufficient. We made a series of point mutants between codon 367 and 382 where eight basic amino acids exist and found that the important amino acid residues for nuclear localization are arginine of codon 367 and lysine of codon 373.

Alternative Splicing of the Novel HMG Box Transcription Factor BBX (HBP2, MHS4R3). *M. Kiehntopf¹, C. Beetz¹, M. Brodhun², J. Schickel¹, T. Deufel¹.* 1) Institut fuer Klinische Chemie, Universitaetsklinikum Jena, Jena, Germany; 2) Institut fuer Pathologie, Universitaetsklinikum Jena, Jena, Germany.

The human homolog of the drosophila bobby sox gene, BBX, was first designated HBP2 and identified by functional complementation of cells unable to undergo DNA replication in fission yeast. BBX contains a high mobility group domain (HMG) with homology to the SRY-box, which was originally identified in the product of the Sex-determining Region on the Y chromosome (SRY) gene. Thus, BBX is considered a member of the SOX family of transcription factors. A role of sox genes has been implied in several genetic disorders such as XY sex reversal, campomelic dysplasia, and Waardenburg-Hirschsprung disease.

By analyzing the chromosomal region 3q13.1, we have identified a gene which we initially designated as MHS4R3. Only later, comparison with database sequences revealed that MHS4R3 and HBP2 are identical genes; we also found that BBX and BBXa are in fact alternatively spliced transcripts of the same gene, differing from MHS4R3/HBP2 in one exon of the coding region.

In our present study, we found that extensive alternative splicing occurs in transcripts of the BBX gene, resulting in a variety of different putative protein sequences. We show that these splice alternatives are differentially expressed in various tissues. The predicted alternative proteins are identical to BBX at the HMG Box-containing N-terminus, but differ at their C-terminus. This is due to a frame shift caused by loss of one exon, and to the combinatorial use of some of the following exons. Interestingly, the predicted number of NLS and NES domains is unique for every splice variant, indicating that it is the C-terminus that determines sub-cellular localisation of the protein variants. Accordingly, transfection of GFP-fusion constructs revealed that splice variants containing certain NLS were localised in the nucleus. Studies are under way to further elucidate the functional role of the different BBX splice variants.

Program Nr: 901 from 2002 ASHG Annual Meeting

KLHL1, the gene expressed from the SCA8 locus, and KLHL4 evolved from the same orthologous gene in fish.
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Spinocerebellar ataxia type 8 (SCA8) is caused by a CTG expansion in an untranslated, endogenous antisense RNA that overlaps the Kelch-like 1 (KLHL1) gene. The normal functions of this antisense transcript and of the protein encoded by the sense transcript are currently unknown. We have performed extensive sequence analysis of human genes homologous to KLHL1 and have determined that KLHL1 is one of a family of paralogous human genes that also include KLHL4 and KLHL5. Mice have genes that are orthologous to each of these three genes, and we recently reported that the mouse *Klh1* gene has a *Klh1*-antisense transcript as well. Our analysis of the *Fugu rubripes* (pufferfish) and zebrafish genomes indicate that these fish species have only two members of this gene family, one of which is orthologous to human KLHL5 and one of which appears to have been derived from a gene that is ancestral to both KLHL1 and KLHL4. We are currently using morpholino (antisense) oligos to knock down the gene products of these orthologous genes in zebrafish embryos in order to determine their normal function, and are determining if natural antisense transcripts are present for either of the zebrafish KLHL orthologs.

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One Step In Situ PCR: An Improved Method of In Situ PCR to Detect Gene Expression on Paraffin Embedding Tissues. X. Chang, R. Yamada, A. Suzuki, S. Tokuhira, K. Yamamoto. SNP research center, The Physical and Chemical Research(Riken),Japan. Yokohama, Shinakawa, Japan.

One Step In Situ PCR: An Improved Method of In Situ PCR to Detect Gene Expression on Paraffin Embedding Tissues X. Chang, R. Yamada, A. Suzuki, S. Tokuhira, K. Yamamoto The Institute of Physical and Chemical Research (Riken), Yokohama, Japan. In situ PCR is becoming a common tool to trace gene expression because of its unique ability to identify rare genes on paraffin-embedding tissue sections. In individual cells, low copy mRNAs are observable with microscopy after they are reverse-transcribed into cDNA (RT) and in situ amplified by PCR. However, this powerful method remains relatively imprecise and its reproducibility is not high. The separation of RT and PCR, the critical steps of the procedure, is mainly responsible for the weak points of the technique, because preparation and handling between RT and PCR steps takes long time and make risks of transcripts to be lost, damaged and/or exposed to the RNase, more likely. Recently, we improved the procedure of in situ PCR by merging RT and PCR into one-step and conducting reaction on PCR oven simultaneously in accordance with methodological principle of one-tube RT-PCR. In our report, we applied the modified method to investigate mRNA expression of aromatase, a critical enzyme for estrogen production using bone marrow tissues. Results showed that aromatase could be detected in bone marrow cells and the signals of aromatase mRNAs are more sensitive and well defined than the regular two step in situ PCR. It is suggested that improved in situ PCR method is exceedingly trustable, practicable and timesaving.

Expression of a splice variant of *TRPM6*, the gene causing Hypomagnesemia with Secondary Hypocalcemia, in mouse testis. *R.Y. Walder, R.E. Swiderski, V.C. Sheffield.* Dept Pediatrics, and Howard Hughes Medical Institute, Univ Iowa, Iowa City, IA.

We recently used positional cloning to identify *TRPM6*, the largest member of the TRP channel protein family, as the gene which causes familial hypomagnesemia with secondary hypocalcemia (HSH)(OMIM 602014). The complete cDNA sequence of *TRPM6* contains 8429 nucleotides, including an open reading frame of 6069 nucleotides. The predicted *TRPM6* protein contains 2022 amino acids and has a calculated molecular mass of 234 kD. The genomic sequence contains 39 exons within 166 kb. *TRPM6* is highly homologous to *TRPM7*, a bifunctional protein which can act as non-selective cation channel and as a serine/threonine protein kinase. Individuals with HSH and mutations in *TRPM6* have defects in the renal and intestinal transport of magnesium. These findings indicate that *TRPM6* plays an important role in magnesium homeostasis in the kidney and in the intestine. Expression data from Northern and RT-PCR analyses of human tissues showed that the full-length transcript, 8.5 kb, is abundantly expressed in kidney and colon, two critical sites of magnesium transport. In mouse tissues, the full-length transcript for *TRPM6*, 8.5 kb, was detected in kidney, and weakly in lung. A smaller transcript, 4.0 kb, was detected with a *TRPM6* probe in mouse testis RNA. We report here the DNA sequence and characterization of the 4.0 kb splice variant of *TRPM6*. Preliminary data suggests that this transcript contains the sequence for the protein kinase, and not the TRP channel domain. Further localization studies of the *TRPM6* transcript have been done by in situ hybridization of testis tissues. These data suggest an independent role for the protein kinase domain in testicular tissue.

Dose-Dependent Shift from Repressor to Activator in a DAX-1 Variant from a Female with Adrenal Hypoplasia Congenita. *B.D. Bowling¹, J.J. Wilson¹, P. Bernard¹, M-B. Dinulos², J. Phelan³, E.R.B. McCabe^{1,3}, E. Vilain^{1,3}.* 1) Human Genetics, UCLA, Los Angeles, CA; 2) Pediatrics, Dartmouth, Hanover, NH; 3) Pediatrics, UCLA, Los Angeles, CA.

The nuclear hormone receptor DAX-1 is responsible, when mutated, for the X-linked disease adrenal hypoplasia congenita (AHC). All known missense mutations impair DAX-1 repression of Steroidogenic Factor-1 (SF-1) transactivation and have been limited to the C-terminal putative ligand binding domain (LBD). An asymptomatic father and an AHC daughter were both discovered to share a DAX-1 sequence variation (C200W). C200W is the first missense mutation found in the N-terminal half of DAX-1 and represents the relatively rare presentation of AHC in a female heterozygous carrier. Co-transfection of equimolar concentrations of C200W DAX-1 with SF-1 into embryonic kidney cells (293T) generated wild-type repression of SF-1 transactivation. However, as the C200W concentration increased, a concomitant loss of functional silencing became apparent. High concentrations of C200W synergistically enhanced SF-1 transactivation to levels exceeding those seen with SF-1 activation alone. We conclude that the unique C200W variant, associated with a normal and an AHC phenotype, has the capacity to convert DAX-1 activity from antagonist to agonist in a dosage-sensitive manner. The clinical variability in this family suggests that additional modifier genes and epigenetic factors are involved. Our studies reveal a multifaceted role for DAX-1 in a complex network critical for the developing reproductive axis.

A T-box containing transcription factor on human chromosome 15q14 identified through a retinoic acid-induction gene trap approach in mouse ES-cells. A. Voigt¹, W. Wurst², M. Weickert¹, D. Schlote¹, I. Hansmann¹, M. Schlicker¹. 1) Institut fuer Humangenetik & Medizinische Biologie, Halle (Saale); 2) Max-Planck Institut, Muenchen, Germany.

Prenatal exposure to retinoic acid (RA) and retinoids (RX) is related with specific congenital anomalies. The molecular mechanism for RA/RX teratogenesis and the genes involved are not well known. To identify RA/RX downstream genes we set up an RA-induced gene trap approach based on mouse ES clones (Forrester et al. 1996). From a sample of trapped ES clones with RA-induced reporter-gene repression 5-RACE-PCR identified a fusion transcript of 1.135 kb in clone EScD1. Screening of cDNA libraries and in silico analysis revealed an approx. 9 kb mouse cDNA. This gene represents a transcription factor containing a T-box and a bHLH Zip-domain. Northern analysis revealed tissue specific expression of this single locus gene with transcripts of varying size. A prominent 9.5 kb transcript is present in spleen, kidney, stomach, lung, brain, testis, ovary, placenta, heart and skeletal muscle. Weaker and smaller transcripts of 7.2 kb were observed in placenta and testis and of approx. 4 kb in testis indicating alternative splicing. A genomic P1 clone isolated contains the complete cDNA. Screening of human cDNA libraries and in silico analysis identified the corresponding human gene with sequence homology >82% to mouse cDNA. BACs were isolated for establishing genomic structure and mapping. The gene contains 24 exons, the same domains as the mouse gene and maps o 15q14, according to FISH. The gene was found to be triplicated in a young proband with mental as well as growth retardation and malformations due to partial trisomy 15q14-q15.

Analysis of the characteristics of nucleotide substitutions at the substrate recognizing regions of Cytochrome P450 2C subfamily enzymes. *S. Kuno, N. Kamatani.* Division of Genomic Medicine, Department of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Tokyo, Japan.

Cytochrome P450 (CYP) superfamily, the oxidate-reduction enzymes, are widely preserved in the biological world. 265 Families of CYP superfamily are known and 69 families have been identified in animals. In 69 families of animals, the members of CYP 1-3 families mainly play the phase I reaction of the metabolism of outer matters to make those harmless. CYP 2C subfamily enzymes metabolize numerous drugs in liver. The genes for four enzymes (2C8, 2C9, 2C18 and 2C19) that are members of this subfamily have been localized in chromosome 10 q24 in human. These enzymes catabolize various toxic compounds. Since the chemical structures of these compounds have varieties, it is reasonable to assume that the substrate recognizing regions of these enzymes undergo diversity-enhancing evolution just as the antigen-binding regions of major histocompatibility complex. To test for the diversity-enhancing evolution, the comparison of the number of synonymous nucleotide substitution (ds) versus that of nonsynonymous nucleotide substitution (dn) between the sequences of CYP 2C enzyme was performed. If dn is more than that of ds, then we suspect that diversity-enhancing evolution might have occurred. CYP 2C subfamily enzymes have six substrate recognizing regions. It was found that dn were enhanced near the substrate recognizing regions by the window analysis with 30 codons. In the comparison between ds and dn in CYP 2C subfamily enzymes, the diversity-enhancing substitutions were observed in the substrate recognizing regions. However, dominance of dn than ds in the short sequence's range may be generated by chance. Therefore, that should be tested for significance. We performed test with the Monte Carlo simulation experiments and it was supported in most cases with significance. Similar results were obtained in rabbit, rat and golden hamster. These results suggest that the substrate recognizing regions of CYP 2C subfamily enzymes have undergone diversity-enhancing evolution due to the selection pressure favoring the diversity opposing against the attack by various toxic compounds.

Monitoring the TP53 gene to detect genetic damage in tissue-engineered skin. *P.E. McAndrew¹, H. Rodriquez², C. O'Connell², M.A. Marino¹.* 1) Applied Genomics, Transgenomic, Inc, Gaithersburg, MD; 2) DNA Technologies Group, Biotechnology Division, NIST.

The purpose of this study was to assess the safety and performance of tissue-engineered materials by monitoring the TP53 gene as a biological safety checkpoint during the manufacturing process. Samples of tissue-engineered skin (TE; TestSkin II) were obtained and included total TE skin (fibroblasts and keratinocytes combined), TE skin (fibroblasts), TE skin (keratinocytes), adult fibroblasts (55 years old), and adult fibroblasts (96 years old). Neonatal fibroblasts and neonatal keratinocytes were included as controls for this study. Denaturing high performance liquid chromatography (dHPLC) was used to scan the TP53 gene for putative mutations. The TP53 gene was chosen as a biomarker for tissue engineered samples because alterations in this gene occur in almost 50% of all human cancers. DHPLC did not detect any putative mutations in the TP53 gene in these tissue-engineered skin samples. Analysis of exons 2 through 11 was performed using nine PCR amplicons and was completed in less than three hours. Three of the samples contain the previously reported 16bp polymorphic insertion in intron 3 (IVS3+40). Heteroduplex peaks were detected in the exon 10 PCR products of the skin keratinocyte and neonatal keratinocyte samples. Sequence analysis confirmed the presence of base changes indicated by DHPLC analysis, both of which are polymorphic changes due to their location. The WAVE® System provides a highly sensitive and rapid technique to screen for genetic alterations in the TP53 gene, and can be used as an effective method to monitor the integrity of tissue engineered samples.

Genomic structure, promoter activity and developmental expression of the mouse homologue of the Machado-Joseph disease gene. *M.C. Costa*¹, *C. Miranda*², *M. Tessaro*², *J. Sequeiros*^{1,3}, *M.M. Santos*^{1,2}, *P. Maciel*^{1,3}. 1) UnIGENE, IBMC, Univ. Porto, Portugal; 2) Dept. Medicine, CHUM, Hôpital Notre-Dame, Montreal, Canada; 3) Dept. Est. Pop., ICBAS, Univ. Porto, Portugal.

Machado-Joseph disease (MJD) is an autosomal dominant neurodegenerative disorder, caused by the expansion of the (CAG)_n tract in the *MJD1* gene. This encodes the protein ataxin-3, of unknown function. We determined the genomic organization of the *MJD1* mouse homologue (*mMJD*), characterized its promoter and studied its expression pattern during embryonic development and in adult mouse tissues. The mouse gene has a similar structure to its human counterpart, with the same number of exons (11), intron and exon sizes comparable to the human, and a TATA-less promoter. To assess the activity of the *mMJD* gene promoter, we constructed CAT reporter plasmids containing different lengths of its upstream region and performed transient transfections into undifferentiated mouse embryonic carcinoma P19 cells. The largest fragment (extending to -1173 bp), showed a significant promoter activity. Minimal activity was observed with the -237 bp construct and maximal activity with the -928 bp construct. Our results also suggest that transcription repressor sequences exist in the regions -559 to -358 and -1173 to -928 bp. Analysis of differentiated P19 cells (neurons and cardiac/skeletal myocytes) showed a stronger activity of the promoter in myocytes, consistent with the bioinformatic identification of myoblast-specific transcription factor binding regions. Immunohistochemical tissue analysis showed that the *mMJD* gene is expressed throughout all developmental stages (embryonic days 11.5-16.5) and in the adult mouse, with a higher expression in brain, heart and skeletal muscle. A muscle-specific 38KDa isoform of the protein was detected by Western blot, whereas an isoform of larger size was apparent in brain. These isoforms were also observed in human tissues, suggesting that they may be of functional relevance. Further characterization of these isoforms and of the regulation of the *mMJD* gene will help to clarify the normal function of ataxin-3 and its physiological role in muscle and brain.

Tissue specific splice variants of the human A-C1 Ha-Ras suppressor gene (HRASLS). *C. Alexander*¹, *S.S. Bhattacharya*², *B. Wissinger*³. 1) Neurodegeneration, Max-Delbrück-Center, Berlin, Germany; 2) Dept. of Molecular Genetics, Inst. of Ophthalmology, London, UK; 3) Molecular Genetics Lab, University Eye Hospital, Tübingen, Germany.

Purpose: A high fidelity physical map consisting of more than 100 PAC clones on chromosome 3q28-q29 had been created as genomic resource. In order to identify novel disease gene candidates, we sequenced this genomic area of ~1MB on chromosome 3q28-q29 flanked by satellite markers D3S3669 and D3S3562. **Methods:** The raw data were edited and reassembled using the Staden Software Package and our own BLAST-able PAC database was established at the HGMP-UK web site. Open reading frames were predicted through NIX analysis and confirmed by BLAST searches of EST databases at the NCBI server. The intron-exon structure of EST clusters was examined by computational alignments of the cDNA and genomic sequence. We performed northern blot hybridization in order to determine tissue specificity of the identified cDNAs. **Results:** Several open reading frames matched with the sequence of Unigene cluster Hs.36761. The underlying gene was recently identified as the A-C1 Ha-Ras suppressor gene (Ito et al., 2001). Northern blot analysis revealed specific expression of this cluster in skeletal muscle (testis material was not included in our blot). One cDNA sequence belonging to the Unigene cluster had been isolated from testis. In this clone, the first non-coding exon of the A-C1-cDNA was replaced by 235 bp of completely unrelated sequence. This 5UTR stretch was found to be divided into three small exons, which were not present in cDNAs derived from other tissues in this Unigene cluster. **Conclusion:** Tissue specific splice variants of the A-C1 Ha-Ras suppressor gene exist. Whereas the coding sequence is the same for all transcripts in all tissues, the 5UTR of a testis specific transcript differs from that of cDNAs in other organs. This finding might hint towards a testis specific control of translation of this particular gene.

Genetic Analysis of Two Sodium Dependent Vitamin C Transporters, *SLC23A1* and *SLC23A2*. H.C. Erichsen¹, P. Eck², J.G. Taylor¹, M. Yeager¹, A. Hughes³, M.A. Levine², S.J. Chanock¹. 1) NCI Advanced Technology Center, Gaithersburg, MD 20877; 2) NIDDK, NIH, Bethesda, MD 20892; 3) Department of Biological Sciences, University of South Carolina, Columbia, SC 29208.

Vitamin C (L-ascorbic acid) is a critical co-factor for at least eight enzymatic reactions and functions as a scavenger of free oxygen radicals. Due to its role as an antioxidant, vitamin C could be involved in the pathogenesis of cancer, cardiovascular disease, diabetes, and stroke. We have previously characterized the human *SLC23A2* gene and now report the characterization of *SLC23A1*. Their tissue expression patterns differ despite a high degree of similarity in amino acid sequence (65%); *SLC23A1* is ubiquitously expressed, *SLC23A2* is restricted to epithelial surfaces involved in bulk transport, such as the intestines and kidney. They share common intron/exon borders but differ in size by ten fold (*SLC23A1* > *SLC23A2*) and in the 5 and 3 UTR regions. Re-sequencing analysis with coding bias was performed in 48 anonymous controls (Caucasians and African Americans) in both (*SLC23A1*, 14 SNPs with no nonsynonymous changes and 2 intronic deletions; *SLC23A2*, 15 SNPs, including 4 nonsynonymous). Genetic variants were identified and haplotypes estimated. Nucleotide diversity for each gene was estimated; they differ between *SLC23A1* (Tajimas $D=2.14$, $P<0.05$) and *SLC23A2* ($D=-1.26$, P , NS), specifically for nonsynonymous SNPs. The ubiquitously expressed gene, *SLC23A1* could be under more constraint than *SLC23A2*. Comparison of genomic organizations for the mouse ortholog *slc23a1* and *slc23a2* shows comparable structure. Human-mouse orthologs demonstrate conservation of size and organization of the genes. Human- mouse and mouse-rat orthologous comparisons show different rates of nonsynonymous substitutions. These data together with an analysis of SNPs suggest that these genes are under different selective pressures. These studies provide the foundation for investigation of the regulation of sodium-dependent ascorbate transport, a mechanism in which differences in activity could bear pleiotropic effects in health and disease, including recommendations for nutritional supplementation.

Transcriptional and genetic re-analyses of CCK promoter repeat suggest that the frequency of the low activity haplotype is decreased in panic disorder. *M. Ebihara, H. Ohba, E. Hattori, T. Yoshikawa.* Lab Molec Psychiatry, Brain Sci Inst, Riken, Saitama, Japan.

Cholecystokinin (CCK), prototypical brain-gut peptide, is a neurotransmitter in brain. It is known that CCK-4, the carboxyl terminal tetrapeptide of CCK has ability to precipitate panic attacks experimentally, suggesting that the possibility CCK could have an important role in the development of panic disorder. To study transcriptional regulation mechanism of the CCK could reveal the possible pathogenesis of panic disorder. We have already reported that a polymorphic short tandem repeat (STR) in the 5' upstream region of CCK promoter. We also classified the STRs into four groups (S, and L) according to their length and -188A/G. The L class allele with -188G was significantly less frequent in panic disorder ($P = 0.0032$, odds ratio = 0.06). To understand the function of STRs and their role in transcription of CCK gene, we subcloned the CCK promoter including STRs into firefly luciferase assay vector, pGL3-basic and transfected into NB1, neuroblastoma cell line. It is suggested that each STR has transcriptional repressor activity. Surprisingly, L class STR and -188G showed decreased promoter activity less than the others. These results suggest that the L - (-188G) haplotype acts as a protective factor against panic by reducing the expression of anxiogenic CCK.

Identification and characterization of a novel member of the bHLH-PAS transcription factor family. D.

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The proximal region of human chromosome 14 has many diseases associated with brain malformations and dysfunctions. In order to identify genes involved in brain development and function, a panel of predicted genes and ESTs mapping to human chromosome 14q13 were screened by LeapFrog PCR analyses to determine whether they were expressed in human fetal brain (21-30 wks). We obtained the cDNA sequence encoding the entire open reading frames of one gene expressed in fetal brain by assembling the cDNA sequences obtained from cDNA library screenings, LeapFrog PCR analyses and database searches. The gene, a novel member of the bHLH-PAS family of transcription factors has 12 exons that span about 867 kb of genomic DNA. The sizes of the exons vary from 77 bp to 1.9 kb and the introns vary from 2.1 kb to 275 kb in size. Two pseudogenes with unknown identity, reside within the second and third introns. The gene encodes two alternative transcripts, which are 1 kb and 3.7 kb in size. There are two possible promoters, one upstream of the first exon and one in the first intron. The smaller transcript encodes a 153 amino acid protein containing the PAS domain. This protein is identical to a 901 amino acid protein encoded by the larger transcript. Northern analyses with probes specific to each transcript showed only the larger transcript was expressed in various parts of the adult brain. Both small and large transcripts are expressed in human fetal brain. The *in silico* expression analyses showed the 1 kb transcript is expressed in the placenta and ovarian tumor, while the 3.7 kb transcript is expressed in the placenta, adult uterus, adult hypothalamus, and several cancer cell lines. Cellular localization studies with the 901 amino acid protein tagged in the C terminus with enhanced green fluorescent protein demonstrated the protein is localized in the nucleus of both COS1 and skin fibroblast cell lines. Members of the bHLH-PAS family of transcription factors have many functions, including neurogenesis and neural function. Our gene is an excellent candidate for central nervous system diseases, such as idiopathic basal ganglia calcification that map to chromosome 14q13.

Functional Role for a Dinucleotide Repeat in *CFTR*. *T. Hefferon, G.R. Cutting.* Inst Genetic Medicine, Johns Hopkins Univ, Baltimore, MD.

Dinucleotide repeats are common in the human genome and are useful as genetic markers in positional cloning studies. It has also been reported that variation in these repeats can modify gene expression and thereby disease. A variable TG tract occurs adjacent to the splice acceptor site of exon 9 of the cystic fibrosis transmembrane conductance regulator gene (*CFTR*). Patient and *in vitro* studies suggest that exon 9 skipping, associated with the common IVS8 5T allele, is affected by variations in the TG tract. Since U and G can basepair in RNA, we speculated that the TG repeat may interfere with splice site recognition by forming a stem-loop structure. To test this, we utilized a minigene comprised of *CFTR* exons 8, 9, and 10 in which the TG tract of 12 repeats was replaced with tracts of equal length which would be more or less likely to form stem-loop structures. Exon 9 skipping occurred in $34.2 \pm 1.0\%$ of transcripts derived from the TG₁₂ minigene (n=11). A CG₁₂ tract, predicted to form a strong stem-loop, resulted in increased exon skipping ($70.6 \pm 1.1\%$ 9- transcripts, n=6). However, TA₁₂ and CA₁₂ tracts, which were *not* expected to form stem-loops, also resulted in increased skipping ($68.1 \pm 1.4\%$ and 100% 9- transcripts, respectively, n=6 each). Furthermore, a randomized tract of equal length produced complete exon 9 skipping (100% 9-, n=6). Thus splicing of exon 9 was not corellated with the likelihood that the dinucleotide tract formed a stem-loop. To test the relationship between the length of the TG tract and exon skipping, constructs were generated with TG tracts of varying lengths. We observed that increasing the length of the TG tract from 12 to 16 TG repeats had no effect on splicing ($34.8 \pm 1.0\%$ 9-, n=6). Interestingly, a reduction in the number of repeats to TG₈ completely abolished exon skipping (0% 9-, n=6). Together, these results indicate that both the nature and length of dinucleotide tracts located near RNA processing signals can influence splicing efficiency.

Identification of two keratin-associated protein (KAP) gene clusters on human chromosome 21. *K. Shibuya, I. Obayashi, S. Asakawa, S. Minoshima, J. Kudoh, N. Shimizu.* Department of Molecular Biology, Keio University School of Medicine, Tokyo, Japan.

We have previously determined the entire genomic sequence of the euchromatic region of human chromosome 21, in which we identified unique repetitive elements on 21q22.3 region and presumed it as a cluster of keratin-associated protein (KAP) genes. KAP is one of major components of human hair fibers. This cluster spans approximately 165 kb and consists of 21 putative genes. Detailed computer analysis of these clustered genes revealed 16 active genes, 2 pseudogenes and 3 relics which were further divided into two sub-families. All the 16 active KAP genes harbor several intragenic repetitive elements and belong to the high sulfur KAP gene family. Transcripts were detected for all the 16 active KAP genes by RT-PCR analysis and their expression was solely in the hair root cells (radix pill cells) and not in any other human tissues examined including skin. Among these 16 active KAP genes, two genes produced a spliced minor transcript as well as the unspliced major transcript, whereas the other 14 genes produced only unspliced transcript, indicating all are intronless genes. Moreover, one of 2 pseudogenes was transcribed, indicating the conservation of active promoter region. Besides the KAP gene cluster on 21q22.3, we also identified another KAP gene cluster on 21q22.11 region. Detailed computer analysis and RT-PCR analysis revealed that this novel cluster spans approximately 800 kb and consists of 30 active genes and 22 pseudogenes. These 30 active KAP genes are classified into three sub-families, however, none of them belongs to high sulfur or ultra-high sulfur KAP genes. Thus, chromosome 21 contains 46 KAP genes in addition to the previously identified 225 genes.

Identify KCNQ1 mutations in patients with family history of lethal cardiac arrhythmia and sudden death. S.

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The long QT syndrome (LQTS) is a cardiac rhythm disorder characterized by prolongation of QT interval on electrocardiograms (ECGs). LQTS patients have higher risk to develop syncope and sudden death, especially under trigger factors. We screened *KCNQ1* gene by SSCP and DNA sequence analyses in 102 families with clinical suspicion of LQTS, Brugada syndrome, idiopathic ventricular tachycardia/fibrillation or aborted cardiac arrest (ACA) or sudden cardiac death (SCD) with unknown causes. *KCNQ1* mutations were identified in 10 families; G269S in transmembrane domain S5, W305X, G314C, Y315C, and D317N in the pore region, A341E and Q357R in transmembrane domain S6, and 1338insC, G568A and T587M in the C-terminus. Five mutations, W305X, G314C, Q357R, 1338insC, and G568A, are novel. A total of 61 gene carriers were identified: 41% showed QTc 460 ms on their resting ECGs. The incidence of syncope/ACA/SCD is 12/2/5, respectively. Of five SCD, three appeared as the first symptom. Compared to affected family members, probands (n=10) had more severe clinical outcome: QTc 493±42 ms with syncope/ACA/SCD as 5/2/4. Stresses were the trigger for 60% of life-threatening arrhythmias, syncope and sudden death. In conclusions, LQT1 phenotype is hidden in some of the gene carriers and sudden death can be the first/last symptom, therefore, genetic testing is important to identify gene carriers in the family members to prevent lethal cardiac arrhythmias and sudden death.

A novel mutation (A227C) of the *SOX9* gene in a patient with campomelic dysplasia without sex reversal. S. Liu, P. Dewing, P. Bernard, B.K. Jordan, E. Vilain. Human Genetics, UCLA, Los Angeles, CA.

Mutations in the human *SOX9* gene leading to haploinsufficiency cause campomelic dysplasia (CD) and autosomal sex reversal in approximately 75% of affected XY individuals. CD is a severe skeletal and cartilage malformation syndrome characterized by bowing of the long bones, hypoplastic scapulae, cleft palate, flat nasal passages, high forehead and low set ears. The incidence varies from .05 to 2 per 10,000 births and most patients with severe CD die neonatally due to respiratory failure. In this study, a 3 year old XY patient born to healthy non-consanguineous parents was presented with CD with normal male genitalia. DNA sequence analysis revealed a C to A mutation in one allele at position 227 (A227C) resulting in an A to E change at amino acid 76 (E76A). DNA sequencing showed that neither of the parents of the affected patient carried the E76A mutation, showing that this was a *de novo*, disease-causing mutation. This novel *SOX9* E76A mutation is the first identified in the 5' region upstream of the HMG binding domain. Remarkably, the mutation in question only caused CD and not sex reversal. The *SOX9* E76A mutant was produced by site-directed mutagenesis and expressed *in vitro*. Electromobility shift assay showed that the E76A mutation did not affect protein binding to a consensus sequence of the *SRY* HMG box, a known binding site for wild-type *SOX9*. The effects of the E76A mutation on DNA bending and transactivation are currently being examined and will add to the understanding of the *SOX9* protein function. The novel and unusual E76A mutation in *SOX9* associated with CD, highlights the importance of the N-terminal region of *SOX9* as a critical area involved in proper bone formation and not in sex reversal.

The human FGF2 level as an important cardiovascular risk factor is strongly influenced by the FGF2 genotype.

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Among the important risk factors for coronary atherosclerosis FGF2 as a potent cytokine is standing out by its function in the proliferative response to vascular injury. Methods: 99 clinically, biochemically and by clinical history well examined patients with angiographically confirmed severe CAD were included (49.9y, SD 8.7, 85 males). The individual FGF2 protein- (ELISA, serum level) and mRNA-expression (competitive RT-PCR, cellular expression of native monocytes from venous blood) were related to the genotype constellation of the exon1-polymorphism C223T of FGF2 located in the 5-UTR in the functionally important ribosome entry site. Results: The patient group was characterized by a protein expression of 13.23 ag/cell and a FGF2-gene expression of 49.19 pg/ml. An association study resulted in a positive correlation of FGF2 mRNA and protein expression ($p < 0.001$). The genotype distribution of the C223T-polymorphism identified 84% of CC carriers, 14% heterozygous individuals (CT) and 2% homozygous mutation carriers (TT). The analyses of the genotype/expression relation revealed significant correlations on transcriptional ($p < 0.01$) as well as on translational level ($p < 0.001$). The highest values of both mRNA- and protein-expression were determined among CC-carriers (14.3ag/cell; 54.4pg/ml serum), whereas CT-carriers showed intermediate values (8.3ag/cell; 23.2pg/ml serum). The lowest values were found among TT-carriers: 3.3ag/cell; 14.5pg/ml serum. Conclusions: The FGF2- serum level can not be used itself as a risk marker in the atherosclerosis development. Apart from pathological processes it is strictly determined by the genetic constellation of the FGF gene, an association which must be considered in the interpretation of the individual coronary risk profile.

Functional analysis of DNA variants in ATM gene by mutagenesis. Z. Yang¹, M. Mitui¹, X. Sun¹, H.H. Chun¹, S. Nahas¹, S.P. Scott², M.F. Lavin², R.A. Gatti¹. 1) Department of Pathology, UCLA School of Medicine, Los Angeles, CA 90095-1732; 2) Queensland Institute of Medical Research, PO Royal Brisbane Hospital, Herston, Brisbane 4029, Australia.

Ataxia-telangiectasia (A-T) is an autosomal recessive disorder involving neurodegeneration, immunodeficiency, chromosomal instability, radiosensitivity, and cancer predisposition. The gene mutated in A-T is called ATM (A-T, mutated). Most ATM mutations found in A-T patients are truncation mutations. However, it has been difficult to determine whether certain missense variants in the ATM gene are mutations or polymorphisms. We have used site-directed mutagenesis to distinguish between functional and non-functional ATM variants, introducing missense substitutions identified in A-T patients into full-length ATM cDNA. We stably transfected the variant expression constructs, P1054R and S707P, into two A-T lymphoblastoid cell lines (AT1ABR and AT7LA) to determine the effect of the DNA change on ATM protein expression and kinase function, as well as on radiosensitivity (measured by the colony survival assay, CSA). Immunoblot results showed that both DNA variants induced ATM protein expression and this protein phosphorylated p53 serine 15 in the A-T LCLs; 'empty' constructs did not. Thus, both S707P and P1054R are polymorphisms, not mutations. CSA results are in progress. Other constructs are being evaluated. Mutagenesis represents an effective approach to distinguishing ATM mutations from polymorphisms.

Cellular dysfunction of mutant SCN5A proteins causing long QT syndrome and Brugada syndrome. *S. You, L. Wu, S. Chen, Q. Wang.* Department of Molecular Cardiology, Cleveland Clinic Foundation, Cleveland, OH.

Mutations in the SCN5A gene encoding the cardiac voltage-gated Na⁺ channel cause long QT syndrome (LQT3) and Brugada syndrome. LQT3 is characterized by a prolonged QT interval on electrocardiograms (ECGs), whereas Brugada syndrome is characterized by right bundle branch block and ST-segment elevation on ECGs. Clinically, both disorders cause ventricular arrhythmias and sudden cardiac death. SCN5A mutations causing LQT3 act by a gain-of-function mechanism, whereas those causing Brugada syndrome are functionally distinct from LQT3 mutations, and act by a loss-of-function mechanism. In this study we investigated the cellular localization of SCN5A mutants causing LQT3 syndrome and/or Brugada syndrome. Expression constructs for five LQT3 mutants, four Brugada syndrome mutants and one Brugada/LQT3 mutant were generated by mutagenesis and each mutant construct was transfected into HEK 293 cells. Immunofluorescent staining and fluorescence microscopy showed that the wild-type SCN5A subunit expressed in HEK 293 cells was localized onto the cell surface. However, SCN5A mutants causing LQT3 including DKPQ, N1325S, R1644H, D1790G and E1784K were localized to both cytoplasm and membrane, with predominant immunofluorescent staining in the cytoplasm. Brugada syndrome mutants including T1620M/R1232W, A1924T, L567Q and 4340delA were also localized onto the cytoplasm and membrane, with predominant immunofluorescent staining in the cytoplasm. Similar results were obtained for 1795insD that causes both LQT3 and Brugada syndrome. Our results suggest that defective surface localization of SCN5A mutants can account for the loss of SCN5A function in Brugada syndrome, and may explain why some LQT3 patients also present with the typical ECG pattern of Brugada syndrome.

Promoter polymorphisms within the IL-1 beta gene correlate positively with extracellular IL-1 beta protein

levels. *A.B. Seymour*¹, *D.G. Perregaux*², *C.A. Gabel*², *L.K. Durham*³, *S.K. Hall*¹. 1) Pharmacogenomics, Pfizer Global Research and Development, Groton, CT; 2) Antibacterials, Inflammation, and Immunology, Pfizer Global Research and Development, Groton, CT; 3) Non-Clinical Statistics, Pfizer Global Research and Development, Groton, CT.

The cytokine Interleukin (IL)-1 is a primary mediator in the pathogenesis of inflammatory diseases such as rheumatoid arthritis (RA), asthma and inflammatory bowel disease (IBD). Previous studies have associated a C3953T variant in exon 5 of the IL-1 gene with increased LPS-stimulated IL-1 protein secretion as well as linking two promoter variants to an increased risk of *H. pylori* induced hypochloridia and gastric cancer (Pociot et al., El-Omar et al.; Hamajima, et. al). We have investigated these polymorphisms within the IL-1 beta gene to test for a genetic contribution to interpatient variance observed with a blood-borne, two-step cytokine production assay that measures extracellular IL-1 levels generated in response to LPS and the combination of LPS/ATP; the nucleotide triphosphate enhances posttranslational processing. Genotypes were determined at three polymorphic loci throughout the IL-1 gene: C-511T, T-31C, and C3953T in each of the 31 subjects to test for association with secreted IL-1 protein levels. Individuals homozygous for the haplotype comprised of T-511, C-31 and C3953 (T-C-C) had a significantly higher mean level of LPS-induced IL-1 secretion compared to individuals with zero or one copy of this haplotype (3.3 ng/ml vs. 1.6 ng/ml, $p=.0028$). Further analysis of these data revealed that the association was driven by the T allele at position -511. This finding was replicated in a subsequent study of 30 subjects with rheumatoid arthritis where we observed -511 TT subjects had a significantly increased amount of extracellular IL-1 beta protein ($p=0.0017$) These data provide evidence that genetic variation within regulatory elements of the IL-1 gene may be associated with altered posttranslational processing efficiency. Finally, the T allele at -511 was observed to be significantly associated with hip osteoarthritis in females. (See Meulenbelt et. al, ASHG 2002).

Pseudoxanthoma Elasticum: Molecular investigations in a consanguineous family further supports the existence of pseudogenes (psABCC6) homologous to the ABCC6 gene. *V. Nau, D.P. Germain.* Department of Genetics, European Hospital Georges Pompidou, Paris, France.

Pseudoxanthoma elasticum (PXE) is an inherited systemic disorder of connective tissue, affecting the skin, the eyes, and the vascular system, with highly variable phenotypic expression. The PXE locus has been mapped to chromosome 16p13.1, and mutations in the ABCC6 gene (previously known as MRP6 or eMOAT), encoding a 1503-aminoacids putative membrane transporter of unknown function, have recently been disclosed as the genetic defect responsible for PXE (Bergen et al., Nat Genet 2000; Germain et al., BBRC 2000; Le Saux et al., Nat Genet 2000; Ringpfeil et al., PNAS 2000; Struk et al., J Mol Med 2000). We have identified a heterozygous missense mutation (G226R) in exon 7 of the ABCC6 gene in a PXE female patient, born from parents who were second cousins. Despite complete scanning of the gene, no further mutation was evidenced. A heterozygous profile was also found in the probands unaffected children, the mutant peak being of much lower amplitude. However, haplotype homozygosity was confirmed at locus 16p13.1, using both extragenic microsatellites (D16S3017 and D16S3060), and intragenic polymorphisms located 3' from mutation G226R (V614A in exon 14, and R1268Q in exon 27), in agreement with the known consanguinity in the family. Taken together, our data indicate that PCR products of exon 7 of the ABCC6 gene were amplified from more than 2 genomic copies. This further supports the existence of ABCC6 pseudogene(s) (psABCC6) highly homologous to the 5' end of the human ABCC6 gene (Germain, J Med Genet 2001). These results will prove invaluable when genotyping patients affected with PXE.

E-box mutation in *RAPSN* promoter region causes endplate (EP) acetylcholine receptor (AChR) deficiency. K. Ohno, J.M. Brengman, A.G. Engel. Dept Neurology, Mayo Clinic, Rochester, MN.

EP AChR deficiency is mostly caused by defects in AChR subunit genes, and, in some cases, in *RAPSN*. *RAPSN* encodes a 43-kDa postsynaptic protein rapsyn that plays an essential role in clustering AChR at the EP. The transcriptional control of *RAPSN* has not been studied to date. The E-box has the consensus sequence of CANNTG and binds to basic helix-loop-helix transcription factors. For muscle-specific genes, the E-box is essential for binding to myogenic determination factors MyoD, myogenin, Myf5, Myf6. Here we report a patient with EP AChR deficiency who is heteroallelic for two *RAPSN* mutations, N88K and an E-box mutation, -27C®G.

We previously reported that N88K has no effect on rapsyn self-association but compromises recruitment of AChR to rapsyn clusters (*AJHG* 70: 875-885, 2002). -27C®G is 27 nucleotides upstream of the major transcriptional start site and mutates the E-box sequence of CAGCTG to GAGCTG. -27C®G is not detected in 400 control alleles. RT-PCR analysis of muscle mRNA indicates that the allele harboring -27C®G is not transcribed.

Luciferase reporter assays reveal that the wild-type *RAPSN* promoter region spanning -586 to +172 drives C2C12-myotube-specific gene expression. Introduction of -27C®G reduces reporter expression 15-fold. Four copies of the wild-type *RAPSN* E-box fused to an SV40 promoter enhance reporter expression 53-fold in C2C12 myotubes, but introduction of -27C®G abolishes this effect.

Electrophoretic mobility shift assays indicate that a nuclear extract of C2C12 myotubes binds more efficiently to the wild-type than to the mutant *RAPSN* E-box. Similarly, in vitro synthesized MyoD and myogenin bind to the wild-type E-box more efficiently than to the mutant E-box.

Decreased association of AChR with rapsyn by N88K and reduced *RAPSN* expression in muscle by -27C®G account for the EP AChR deficiency in our patient. To our knowledge, this is the first report of a human E-box mutation.

A POSSIBLE ASSOCIATION BETWEEN THE T-287C POLYMORPHISM IN THE 5' REGULATORY REGION OF THE TISSUE FACTOR PATHWAY INHIBITOR GENE AND VENOUS THROMBOSIS. A.A.

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Tissue factor pathway inhibitor (TFPI) is the principal regulator of tissue factor induced blood coagulation pathway. It inhibits FXa directly and inhibits TF-FVIIa indirectly by formation of the FXa/TFPI/TF/FVIIa quaternary complex. We have previously shown that low levels of total TFPI may be associated with deep venous thrombosis (DVT). DNA samples from 96 blood donors were screened for polymorphisms in the 5' flanking region of the TFPI gene by denaturing high performance liquid chromatography (DHPLC) using the Transgenomic WaveTM Analysis System. Samples showing different chromatogram patterns were selected for sequencing. A CT transition at -399, and a TC at -287 were identified. T-287C polymorphism was genotyped in 134 DVT patients who had low levels of TFPI and 120 sex-age matched as a control group. The 287C allele was found to be present at a significant higher frequency in the DVT patients with low total TFPI levels than control samples ($p=0.023$). Since we have previously shown that low total serum TFPI levels are associated with DVT in the same group of patients, we are currently investigating the effects of the promoter polymorphisms on TFPI gene expression using a luciferase reporter gene expression assay.

Transcriptional activity of variants of the Z-DNA forming NRAMP1 promoter repeat polymorphism. *M.G.*

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Background: NRAMP1 is thought to be involved in iron homeostasis. Previous studies indicated that allele 2 [t(gt)5(gt)5(gt)10] of a Z-DNA forming repeat polymorphism in the promoter region is associated with infectious disease susceptibility and allele 3 [t(gt)5(gt)5(gt)9] with autoimmune disease susceptibility. Aim: To determine the effect of the various repeat polymorphism alleles on NRAMP1 expression. Materials and methods: The variants were cloned in a luciferase-reporter vector (pGL2) and transfected into U937 and THP-1 cell lines using FUGENE 6. Cells were incubated in the absence and presence of exogenous stimuli, including interferon-g, bacterial lipopolysaccharide, bacterial lipopolysaccharide plus interferon-g, and ferric ammonium citrate. Luciferase activity in harvested cells was determined luminometrically. Results and Discussion: Six promoter variants were studied, including four previously described variants [alleles 2 and 3, allele 4 (t(gt)5(gt)5(gt)4) and allele 5 (t(gt)4(gt)5(gt)10)] and two novel variants [allele 6 (t(gt)5(gt)5(gt)6) and allele 7 (t(gt)5(gt)5(gt)8)], and one previously described base pair substitution (-237C/T). Ferric ammonium citrate caused a five-fold enhancement of luciferase reporter gene expression for allele 3 with U937 cells. Interferon-g and bacterial lipopolysaccharide caused a ten-fold enhancement of gene expression for allele 5 and a five fold-enhancement for the addition of interferon-g for the -237C/T polymorphism in THP-1 cells. Conclusion The alleles differ in their ability to drive gene expression between constructs and cell lines. These results support the hypothesis that this functional repeat polymorphism could contribute directly to the association of variants with infectious or autoimmune disease.

CTCF binding sites upstream of *GTL2/MEG3* form a methylation sensitive putative insulator in the imprinted domain of 14q32. *L.G. Shaffer*¹, *B. Kwabi-Addo*¹, *K.J. Coveler*¹, *Y.Q. Wu*², *V.R. Sutton*¹. 1) Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Clinical Cancer Prevention, M.D. Anderson Cancer Center, University of Texas, Houston, TX.

Genomic imprinting is an epigenetic phenomenon in which the activity of a gene is reversibly modified depending upon the parent of origin. This leads to unequal expression from the maternal and paternal alleles of a diploid locus. Regulation of one imprinted region, *IGF2/H19* on 11p15, is mediated through 7 CTCF binding sites located upstream of *H19*. At least two imprinted genes have been identified within the region containing *GTL2/MEG3* and *DLK1* on 14q32. Methylation of DNA sequences is one mechanism of regulating gene expression. Although it has been demonstrated that *GTL2/MEG3* is expressed exclusively from the maternal allele, the regulatory elements that modulate its allele-specific transcription are poorly understood. We identified six putative CTCF binding sites within the region immediately upstream of *GTL2/MEG3*. Using an electrophoretic mobility shift assay, we demonstrated CTCF binding to each of these six sites. Mutated or methylated CTCF binding sites showed reduced CTCF binding affinity compared to the wildtype sites. We demonstrated CTCF binding to our putative sites using a chromatin immunoprecipitation (ChIP) assay. One of these CTCF binding sites contains a CpG dinucleotide within a *SacII* restriction endonuclease site. Further analysis of this site using restriction enzyme digestion followed by Southern hybridization in seven individuals with uniparental disomy 14 (UPD14) showed differential methylation patterns between maternal and paternal homologues. This site was unmethylated on the maternally inherited chromosomes 14 and methylated on the paternally inherited chromosomes 14. Thus, we have demonstrated parent-specific methylation of sequences 5' of *GTL2/MEG3*. Based on our observations, we speculate that this CTCF-binding region may provide a mechanism for the transcriptional regulation of the imprinted genes *GTL2/MEG3* and *DLK1*, in a manner similar to the regulation of the *IGF2/H19* domain on 11p15.

Fabry Disease: Identification/Characterization of Novel Double Mutations in the α -Galactosidase A Gene. M.

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Fabry disease, an X-linked inborn error of glycosphingolipid catabolism, results from the deficient activity of the lysosomal exoglycohydrolase, α -galactosidase A (α -Gal A). To date, over 300 mutations have been identified in the α -Gal A genes of affected males with the classical or cardiac variant phenotypes. Here, we report three unrelated classically affected males who had two missense mutations in their α -Gal A alleles: C172G/D313Y, A143T/Q312H, and D264Y/V269M. Screening of 300 normal X-chromosomes indicated that only D313Y was a polymorphism, with a frequency of 3%. In retrospect, two other classical Fabry families also had the D313Y. To determine the disease causing mutations in the probands, the single and double mutated constructs were expressed in COS-7 cells, and their subcellular localization was determined by immunofluorescence. Compared to that of the wild type, the A143T, D313Y, and V269M constructs had ~40%, ~60%, and ~10% residual enzymatic activity, respectively. The D313Y and V269M mutant enzymes were sorted to the lysosomes, while A143T was detected in the ER and lysosomes. In contrast, Q312H, C172G, D264Y, and the double mutated proteins, which had no or <5% enzymatic activity, were retained in the ER. The relative proportions of the 55k Da precursor and 46k Da mature subunits for each mutant observed by Western blot analysis were consistent with the immunofluorescence results. Molecular modeling revealed that A143T, D313Y, and V269M did not alter the active site, whereas C172G and D264Y did. Q312H, opposite the active site, caused misfolding, while D313Y was active. These are the first structure-function correlations for human α -Gal A, and provide further understanding of the disease causing lesions. Furthermore, these studies highlight the importance of expression analysis of each base substitution for the accurate distinction of mutations and polymorphisms.

Comprehensive expression profile of a newly cloned RNA binding protein gene. *Y. Wang*¹, *G. Lu*³, *GL. GL. Wilson*¹, *L. Tan*¹, *J. Gu*², *T.-J. Chen*¹. 1) Medical Genetics, University of South Alabama, Mobile, AL; 2) Biomedical Sciences, University of South Alabama, Mobile, AL; 3) Genzyme genetics, NY.

Newly cloned human CPEB gene encodes an RNA binding protein, which can specifically recognize the cytoplasmic polyadenylation element (CPE) on mRNA. In mouse, and *C. elegans*, CPEB has been shown to play roles in both polyadenylation-dependent translational activation and CPE-directed translational repression. In mouse brain, CPEB is highly expressed in neuronal cell bodies and synapto-dendritic compartments. Its translational regulation function in response to synaptic stimulation has been shown to be a very important step in synaptic plasticity, which is the basis for the learning and long-term memory storage. We attempted to explore the functions of CPEB in human, since defects in this gene may lead to mental retardation. The cloned cDNA of hCPEB is about 2000 bp. It encodes a 486 amino acid protein with a predicted molecular weight of 54 KD. Its amino acid sequence has an overall 95% identity to mouse CPEB with particularly high homology on the two RNA recognition motifs and the zinc finger domain. The hCPEB comprehensive expression profiles from 22 tissue types were determined by RT-PCR. The first-strand cDNAs from each tissue were first tested and normalized using b-actin cDNA as an internal standard. Human CPEB cDNA and the b-actin cDNA were co-amplified in the same tube. Results from this RT-PCR assay revealed that hCPEB has high-level expression in brain, ovary and testis. However, very low expression in liver, stomach, and skin. Heart, kidney, skeletal muscle, thyroid, adrenal gland, and pancreas have moderate levels of expression. Its expression in the fetal brain is much lower than in the adult brain. In situ hybridization revealed that hCPEB expression is limited to neurons in the human brain. Specific labeling of hCPEB was detected in the cytoplasm of the neurons in the cortex but absent from the nuclei and the extracellular space. Most of the neurons were positive. Our results suggest that hCPEB has a similar expression profile to mouse CPEB and may have similar functions in translational regulation in human brain.

INFLUENCE OF THE APOLIPOPROTEIN POLYMORPHISMS ON PLASMA LIPID LEVELS IN

BRAZILIAN CHILDREN. *E. FRANCA*¹, *J.G.B. ALVES*², *A.S. ARAUJO*³, *M.H. HUTZ*¹. 1) Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil; 2) Instituto Materno Infantil de Pernambuco, Recife, Pernambuco, Brazil; 3) Hemocentro de Pernambuco, Recife, Pernambuco, Brazil.

Genetic studies in adults have shown that common polymorphisms in gene coding for apolipoproteins are significant determinants of plasma lipid levels variation. The influence of the allelic variation at the APOE gene locus (E*2, E*3 and E*4) on plasma lipoprotein parameters have been well evaluated. This influence have not been clearly established for others apolipoproteins. We have studied five apolipoprotein polymorphisms, including APOE/HhaI, APOA-I/MspI-75bp, APOA-I/MspI+83bp, APOA-IV/HinfI and APOA-IV/PvuII in 414 (221 boys and 193 girls) unrelated children aged 5 to 15 years from Pernambuco, a Brazilian Northeast state. The frequencies of the APOE alleles (E*3, E*4 and E*2) were 0.77, 0.17 and 0.06, respectively. The rare allele(A) frequency for the polymorphism APOAI/MspI was 0.18 at -75bp and 0.11 at +83bp. The frequencies of the 347Ser and 360His alleles of the APOA-IV were 0.14 and 0.03, respectively. The influence of the alleles on lipid traits was evaluated by ANOVA test or Kruskal-Wallis test. The allele E*2 is associated with lower levels of Total ($p<0.001$), LDL-C ($p<0.001$) and Total/HDL-C ratio ($p<0.001$). Conversely, the E*4 allele is associated with higher levels of these lipids. No significant association was observed between plasma lipids and the APOA-I/MspI -75bp polymorphism. However, girls heterozigous(+/-) for loss of the site APOA-I/MspI+83bp had higher ($p<0.026$) HDL-C levels than girls homozigous(++). The polymorphism APOA-IV/360PvuII was not associated with lipid concentrations. In the APOA-IV/347HinfI polymorphism the Ser allele was significantly associated in girls ($p<0.016$) with increased HDL-C when compared to Thr allele. These data indicate that the effect of the apolipoprotein polymorphisms on plasma cholesterol levels also can be observed in children.

Human Glycerol Kinase (GK): Tissue-Specific Expression Patterns of Two Alternatively Spliced mRNAs from the Xp21 GK gene. *R.H. Ohira*¹, *K.M. Dipple*², *E.R.B. McCabe*^{1, 2, 3}. 1) Departments of Human Genetics, and; 2) Pediatrics, David Geffen School of Medicine at UCLA; 3) UCLA Molecular Biology Institute, Los Angeles, CA 90095.

Glycerol kinase (GK) phosphorylates glycerol to glycerol 3-phosphate, an intermediate in the formation of glycerolipids. Mutation of GK causes GK deficiency (GKD), an X-linked inborn error of metabolism. There are two alternatively spliced forms of GK: with exon 18 (GK+EX18) in human fetal brain and testes (Sargent et al, Hum Molec Genet 3:1317, 1994) or without exon 18 (GK-EX18) found in liver (Guo et al, Nature Genet 4:367, 1993). Our purpose was to determine the expression profiles of the GK mRNAs in different tissues. Reverse transcriptase-polymerase chain reaction (RT-PCR) using primers to the human Xp21 GK sequence within exon 16 and the 3'-untranslated region (3'UTR) resulted in a 280 bp product for GK+EX18 or 193 bp product for GK-EX18. RT-PCR was conducted on human fetal brain, adult brain, liver, kidney and testis poly-A RNA, as well as on total RNA extracted from cell lines including HepG2 (human liver), WRL-68 (human fetal liver), A-549 (human lung), Cos-7 (monkey kidney), H-4-II-E (rat liver), McA-RH7777 (rat liver), NMuLi (mouse liver), a lymphoblastoid cell line (LBL) derived from a GK-deleted patient (131509-1), and two LBLs from normal individuals. Human liver and kidney only expressed the GK-EX18 transcript. However, human fetal brain, adult brain, testis, the two normal LBLs and the WRL-68 cell line expressed both transcripts, with the GK+EX18 transcript being more prevalent. HepG2 expressed both transcripts with equal intensity. The non-human cell lines (A-549, Cos7, H-4-II-E, McA-RH7777 and NMuLi) and the GK-deleted LBL did not have either PCR product, indicating that the RT-PCR was human-specific and Xp21 GK-specific. We conclude that the two alternatively spliced human GK mRNAs are differentially expressed and speculate that these differences in expression patterns suggest specific and possibly divergent functions for the GK+EX18 and GK-EX18 proteins.

Methylation analysis of the promoter region in fragile X males with hypermethylated, full mutation alleles. *F. Tassone*¹, *P. Sahota*¹, *N. Pleasant*², *C. Laird*², *P. Hagerman*¹. 1) Dept Biological Chemistry, Univ California, Davis, Davis, CA; 2) Department of Zoology, University of Washington, Seattle, Washington.

Methylation of the CpG islands in housekeeping genes has been shown to cause their transcriptional silencing. Expansion of the trinucleotide repeat CGG, located within the 5'UTR of the Fragile X mental retardation 1 gene (FMR1) leads to hypermethylation of -CpG dinucleotides within and upstream of the repeat element. As a consequence the gene becomes silenced and no FMR1 protein is produced. Although methylation of the FMR1 gene is generally accompanied by the absence of FMR1 transcript, we have recently reported a group of male carriers of hypermethylated full mutation alleles who escape the silencing process to varying degrees. Thus, hypermethylation, normally defined as resistance to enzyme cleavage, does not always lead to gene silencing. In order to determine if the absence of specific CpG methylation events within the CpG island region is associated with continued gene activity for hypermethylated full mutation alleles, we have used bisulfite-coupled sequencing to map the positions of methylated/unmethylated CpG dinucleotides in the genomic DNA from twelve males with hypermethylated FMR1 alleles. The region analyzed is, upstream of the CGG repeat element, about 450 bp in length, and contains 52 CpGs. Here, we report progress in our analysis of all methylation pattern variants in this region of the FMR1 promoter. Based on a total of over 400 clones from twelve males (about 35 cloned alleles/male), our results to date indicate that even within heavily methylated alleles, there exist distributed positions that remain at least partially unmethylated; that is, methylation patterns are not all-or-none.

Ferroxidase Activity of Frataxin, the Protein Deficient in Friedreich Ataxia. *O. Gakh*¹, *S. Park*¹, *S.M. Mooney*¹, *H.A. O'Neill*¹, *H.K. Nichol*², *I.J. Pickering*², *G.N. George*², *A. Mangravita*³, *G.C. Ferreira*³, *G. Isaya*¹. 1) Dept. of Pediatric & Adolescent Medicine and Biochemistry & Molecular Biology, Mayo Clinic, Rochester, MN; 2) Stanford Synchrotron Radiation Laboratory, SLAC, Stanford, CA; 3) Dept. of Biochemistry & Molecular Biology, University of South Florida, Tampa, FL.

The mitochondrial protein frataxin is required for cellular iron homeostasis but its mechanism of action is not yet known. We showed previously that in the presence of Fe(II) recombinant yeast frataxin (mYfh1p) assembles into a 840 kDa complex that accumulates iron in soluble form (*Adamec et al. 2001; Gakh et al. 2002*). Iron cores of 2-4 nm are detected by STEM-EDS, and high-resolution EXAFS to $18 \text{ K } \text{\AA}^{-1}$ demonstrates that they resemble 6-line ferrihydrite, the principal biomineral found in ferritin. The mechanism of iron incorporation into the mYfh1p complex has been investigated. The protein binds Fe(II) and regulates its oxidation to Fe(III) in two phases. The first phase corresponds to the assembly of an oligomeric species that catalyzes iron oxidation with a Fe(II)/O₂ stoichiometry of ~2 consistent with ferroxidase activity. The second phase is characterized by Fe(II)/O₂ stoichiometry of ~4 consistent with autoxidation. During stepwise assembly of the complex, ferroxidation is rapidly overcome by a slow autoxidation phase during which the remaining Fe(II) is loosely bound to the protein and can be readily donated to a chelator or the mitochondrial enzyme ferrochelatase to synthesize heme. Assembly is completely inhibited under anaerobic conditions indicating that mYfh1p senses the presence of both Fe(II) and O₂. Frataxin may therefore represent a mechanism to limit participation of Fe(II) in radical-generating reactions and at the same time promote mitochondrial iron metabolism. Supported by NIH/NIA AG15709.

Identification and characterization of human MNMA gene related to mitochondrial tRNA modification. *Q.F. Yan, R.H. Li, X.M. Li, M.X. Guan.* Dept Human Genetics, Children's Hosp Medical Ctr, Cincinnati, OH.

The nucleotide modification in tRNA plays a pivotal role in the fidelity of translational process. The defects in nucleotide modification have often been observed in the mutated mitochondrial tRNAs associated with human diseases. Recently, bacterial *mnmA* encodes the 5-methylaminomethyl-2-thiouridylate methyltransferase, which has been shown to be a component of tRNA modification pathway. Here we report the identification and characterization of human MNMA homolog. Based on the available human EST sequence homologous to bacterial *mnmA*, we have isolated and characterized the human MNMA cDNA and elucidated the genomic structure of this gene. This gene encodes a predicted protein of 47,714 daltons with a typical mitochondrial targeting sequence. The amino acid alignments of the human MNMA with other identified homologs from different species revealed an extensive evolutionary conservation in amino acids and size. This gene, localized in chromosome 21, is composed of 11 exons, ranging from 54 bp to 498 bp and 10 introns varying from 89 bp to 5,317 bp. MNMA is ubiquitously expressed in various tissues, but with markedly elevated expression in tissues of high metabolic rates. We also showed that Gtpbp3 localizes in the mitochondrion by immune-staining with specific antibodies and analyzing with a confocal microscopy. These observations imply that human MNMA is a structural and functional homolog of bacterial *mnmA*, thereby playing a role in the mitochondrial tRNA modification and protein synthesis.

Mice deficient in pyrin, the FMF protein, show increased sensitivity to endotoxin through a new pathway regulating IL-1 activation and apoptosis. *J.J. Chae*¹, *J. Cheng*², *H. Komarow*¹, *G. Wood*¹, *N. Raben*³, *P.P. Liu*², *D.L. Kastner*¹. 1) Genetics and Genomics Branch, NIAMS, NIH, Bethesda, MD; 2) Genetics and Molecular Biology Branch, NHGRI, NIH, Bethesda, MD; 3) Arthritis and Rheumatism Branch, NIAMS, NIH, Bethesda, MD.

Familial Mediterranean fever (FMF) is a recessive disorder of inflammation characterized by episodes of fever with serositis or synovitis. To facilitate studies of pyrin's function, we have used gene targeting to produce mice with a disruption of *Mefv*. These mice exhibit exaggerated body temperature responses to endotoxin (LPS) relative to wild-type littermates. To explore the mechanism of endotoxin sensitivity in pyrin-deficient mice, we studied protein expression by Western blotting in peritoneal macrophages. In peritoneal macrophages from normal animals, mouse pyrin (mpyrin) was induced by LPS and by the anti-inflammatory cytokines IL-4 and IL-10. Induction of mpyrin inhibits IL-1 processing, as evidenced by the fact that peritoneal macrophages from pyrin-deficient mice, but not their wild-type littermates, produced large amounts of mature (cleaved) IL-1a and IL-1b when treated with IL-4 and LPS. Moreover, when the RAW264.7 mouse monocytic cell line was transfected with full length *Mefv*, LPS-induced mature IL-1b was markedly diminished. We found that this effect is likely to be mediated through mASC, an adaptor protein containing an N-terminal pyrin domain (PYD) and a C terminal CARD. As has recently been shown in the human, we found that mpyrin binds mASC through homotypic PYD interactions. We further observed that mASC binds procaspase-1 (interleukin 1 converting enzyme), and, when transfected into RAW264.7 cells, mASC mediated processing of procaspase-1 into active subunits capable of cleaving IL-1b. Pyrin appears to inhibit this pathway, since, in pyrin-deficient mice, LPS induced caspase-1 activation was much greater than in wild-type littermates. We also found evidence for defective apoptosis in the peritoneal macrophages of pyrin-deficient mice. Taken together, these results suggest that pyrin acts as an anti-inflammatory molecule by inhibiting IL-1 production and by permitting monocyte apoptosis.

Analysis of the survival of motor neuron (SMN1) gene mutations in Spinal Muscular Atrophy through models. *N. Owen*¹, *U. Monani*², *S. Squire*¹, *M. Sendtner*³, *A. Burghes*², *K.E. Davies*¹. 1) Dept Human Anatomy/Genetics, University of Oxford, Oxford, UK; 2) Dept. of Molecular Genetics, Ohio State University, Columbus, Ohio; 3) Dept. of Neurology, University of Würzburg, Würzburg, Germany.

Childhood onset Spinal Muscular Atrophy (SMA) is a common autosomal recessive disorder characterised by the loss of a motor neurons and proximal muscle weakness. The Survival Motor Neuron (SMN1) gene is mutated/deleted in the majority of SMA patients and undergoes little alternative splicing. However expression of the copy gene SMN2 results in predominately alternatively spliced transcripts lacking exon 7 or 5. Therefore, in patients the amount of full length SMN protein is markedly reduced. The SMN protein has been demonstrated to be involved in an increasing number of cellular processes, including; RNA processing, snRNP biogenesis, and transcriptional activation, although these functions do not elucidate why this disease has such a specific target tissue.

To further understand the effect of patient mutations and the alternative splicing of SMN2 we have characterised mutant SMN proteins in a number of different cell lines. To specifically characterise the loss of exon 5 of SMN2 transcripts, the second most frequently alternatively spliced exon, we have generated transgenic mice expressing human SMN25. A second transgenic line utilises the E134K patient mutation, a mutation known to alter the interaction of SMN with Sm core proteins. The function of these transgenes was analysed by crossing onto the *Smn* null background..

Analysis of our model system in the yeast has lead to the identification of a important missense mutation in a highly conserved region of the SMN peptide. This domain is analogous to a sequence in human exon 5, spliced in some SMN2 transcripts. To date, the only proteins known to interact with this region of SMN are the profilins (PFNI and PFNII). In vitro binding assays have been carried out to investigate the effect of mutation of SMN on binding to profilins.

Generation of a mouse model of Willams-Beuren Syndrome. *T. Onay*¹, *L. Osborne*², *L-C. Tsui*^{1,3}. 1) The Department of Genetics, the Hospital for Sick Children; 2) the Department of Medicine, University of Toronto; 3) and the Department of Molecular and Medical Genetics, University of Toronto, Toronto, Ontario, Canada.

Willams-Beuren Syndrome (WBS) is an autosomal dominant multisystem developmental disorder caused by haploinsufficiency for genes in a 1.5Mb region of human chromosome 7q11.23. The commonly deleted region encompasses about 18 genes, which include the elastin gene (ELN) that is known to be responsible for the cardiovascular lesions; however, the genes contributing to the other symptoms remain undetermined. Recent studies have narrowed the critical region spanning eight genes including ELN, but further refinement has been difficult due to the paucity of additional deletions. It is therefore unclear whether the complex phenotype is the result of deletion of contiguous genes or a subset of them. We aim to study the effects of multiple gene deletions and to use gene complementation to rescue aspects of the resulting phenotype. A mouse model of WBS is being created with a large deletion spanning seven genes from the critical region with the exclusion of ELN. Our approach is based on the Cre/loxP site specific recombination system. To enable this work, a detailed physical map for the telomeric region of mouse chr. 5, homologous to the human 7q11.23 region, has been constructed. There is remarkable conservation of gene content and order between human and mouse for the region. Accordingly, a proximal 5Hprt-loxP cassette was targeted into the *Gtf2i* gene in mouse ES cells, with the replacement of a 1073bp fragment containing part of exon30 and exon31. 42 of correctly targeted ES cell clones have been identified at a targeting frequency of 25% and two of these lines are being used to insert a second loxP cassette into the *Limk1* gene, which is located about 500Kb telomeric to *Gtf2i*. Cell lines with properly juxtapositioned cassettes will be used to generate the intended, large deletion. Correctly rearranged ES cells will be used to generate hemizygotously and homozygotously deleted mutant mice to help understand the pathogenic basis of the WBS phenotype. This mouse model should provide insight into the mechanisms leading to growth and developmental disturbances observed in the WBS patients.

Palmitoyl-protein thioesterase 1 (PPT1) function in *Drosophila*: Towards a molecular understanding of infantile neuronal ceroid lipofuscinosis. *C.A. Korey, M.E. MacDonald.* Molecular Neurogenetics Unit, Massachusetts General Hospital, Bldg 149, 13th Street, Charlestown, MA 02129.

The most common pediatric neurodegenerative diseases (1 in 12,500 births) are a set of primarily recessive disorders termed Neuronal Ceroid Lipofuscinoses (NCLs). The individual NCLs are classified by their characteristic membrane/protein inclusion pathology and age of onset. The symptoms of the disorders include loss of vision, motor dysfunction, intellectual decline, and seizures. Genetic analysis of the NCLs has identified 8 loci, CLN1-8, that are associated with the differing ages of onset of the disorders. CLN1 was identified as the defective gene in infant onset NCL (INCL) and it encodes the enzyme palmitoyl-protein thioesterase 1 (PPT1). PPT1 is one of the enzymes that catalyze the de-palmitoylation of protein substrates at specific cysteine residues. Its biochemical function suggests that the loss of PPT1 may cause defects in signaling pathways and membrane/protein trafficking through the misregulation of the palmitoylation state of specific proteins, such as PSD95, Ras, SNAP 25, and the neuronal protein GAP-43. This palmitoylation event is dynamic and may play a role in correct membrane trafficking, protein localization and protein-protein interaction. Consistent with this and the fact that neurons are preferentially affected, recent work suggests that CLN1/PPT-1 is associated with synaptic vesicles and synaptosomes in neuronal cell culture. A search of the *Drosophila* genome sequence has found that only the infant onset (CLN1) and the juvenile onset (CLN3) proteins have homologs in the fruit fly. The use of over-expression in *Drosophila* has proven to be a powerful method of understanding gene function and identifying companion signaling components. For example, components of both the dRac1 and dRas signaling pathways were identified through their over-expression in the developing adult visual system. We have developed an over-expression assay, using the GAL4-UAS system, to begin to elucidate the normal cellular functions of *Drosophila* CLN1, thus shedding light on the INCL disease mechanism.

Gene expression in spinal cords of Spinal Muscular Atrophy mouse models. *C.M. Armour^{1,2}, S. Balabanian¹, S. Hayward-McClelland¹, N.H. Gendron¹, A.E. MacKenzie^{1,3,4}*. 1) Molecular Genetics Laboratory, Research Institute, Children's Hospital of Eastern, Ottawa, Ontario, Canada; 2) Department of Clinical Genetics, Children's Hospital of Eastern Ontario, Ottawa, Ontario, Canada; 3) Department of Pediatrics, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada; 4) Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada.

Spinal Muscular Atrophy (SMA), an autosomal recessive disorder characterized by symmetrical muscle wasting due to motor neuron death, is caused by deletions of the SMN1 gene (survival of motor neuron). A number of SMN interacting partners have been identified and SMN functions proposed, chiefly associated with the machinery which processes hnRNA to mRNA. However, the mechanism causing the strikingly specific motor neuron degeneration remains unknown. In an effort to answer this question, we are establishing a detailed profile of mRNA changes caused by SMN depletion in spinal cords of mice with mild SMA (Schrank et al., 1997). Mice that are *Smn* haploinsufficient (*Smn* +/-), lose 50% of their motor neurons by 6 months and are used as a model for human type III SMA. RNA from spinal cords of 5 week old (*Smn* +/- and *Smn* +/+) mice have been used for representational difference analysis (RDA) and DNA microarray hybridization (Affymetrix). RDA (subtractive hybridization combined with selective PCR amplification) has identified approximately 250 transcripts which are either enriched or depleted in *Smn*+/- spinal cords when compared with *Smn* +/+ wild type siblings. We have identified approximately 100 significant changes in the Affymetrix arrays. We have further confirmed these changes in expression by semi-quantitative RT-PCR. Clones obtained by both these methods can be classified into major groups including cell cycle regulation, cell differentiation and proteolytic degradation.

SUBCELLULAR LOCALISATION OF PYRIN PROTEINS CARRYING DIFFERENT FMF MUTATIONS, EXPRESSED IN HELA CELLS. *S. Papin, C. Cazeneuve, P. Duquesnoy, I. Jeru, S. Amselem.* Genetics, INSERM U468, Creteil, France.

Mutations in MEFV, a gene encoding the pyrin, are associated with familial Mediterranean fever, a genetic condition characterised by febrile episodes of serosal inflammation. The function of pyrin is still unclear, although recently, the ASC (Apoptosis Speck protein containing a CARD) protein has been shown to interact with the pyrin domain of pyrin, both proteins being colocalized in specks, suggesting that pyrin may play a role in regulation of apoptosis. To study the influence of MEFV mutations on the subcellular localisation of pyrin, we transiently expressed in HeLa cells wild type pyrin-GFP (pyrin fused to GFP protein) or pyrin-GFP harbouring either the M694V, M694I, V726A, M680I or E148Q mutation; we also co-expressed the different forms of pyrin-GFP (wild type and mutated forms) together with ASC (fused to a V5 tag). The ASC protein was revealed using a mouse anti-V5 antibody and secondary anti-mouse antibody coupled with the red fluorescent molecule Cy3. In cells transfected with pyrin, we found that all the mutated pyrin-GFP proteins localised exclusively in the cytoplasm, with a pattern similar to the one observed with the wild type pyrin-GFP. Cells expressing a low level of recombinant pyrin exhibited a labelling homogeneously distributed over the entire cytoplasm, whereas cells expressing high levels of pyrin presented often with highly fluorescent bodies over a faint and homogeneous cytoplasmic labelling. In cells co-expressing ASC and pyrin-GFP, the green fluorescence due to pyrin-GFP and the red fluorescence corresponding to ASC were concentrated in specks, whatever the pyrin-GFP construct (wild type or mutated forms). These results suggest that these MEFV mutations do not affect the subcellular localisation of pyrin. In addition, whatever the consequences of the mutant pyrin protein in the regulation of apoptosis, it is tempting to speculate that these consequences do not result from an absence of ASC/pyrin colocalisation.

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Association of syncoilin and desmin: linking intermediate filament proteins to the dystrophin-associated protein complex. *E. Poon, E.V. Howman, J.E. Britton, S.E. Newey, A.C. Potter, K.E. Davies.* Department of Human Anatomy and Genetics, Oxford University, Oxford, Oxfordshire, UK.

Duchenne muscular dystrophy is an X-linked muscle-wasting disease that affects 1 in 3500 boys and is caused by mutations in the dystrophin gene. In skeletal muscle, dystrophin associates with a number of proteins to form the dystrophin-associated protein complex (DAPC). We recently identified a novel protein, syncoilin, through its interaction with alpha-dystrobrevin, a member of the DAPC. Syncoilin is a putative intermediate filament (IF) protein and has been shown to interact with desmin, the main IF protein in muscle. Syncoilin is upregulated in human and mouse myopathies, suggesting a role in maintaining muscle fibre integrity. Through its interaction with alpha-dystrobrevin and desmin, syncoilin provides a link between the DAPC to the cytoskeleton. Syncoilin is therefore an ideal candidate gene for muscular dystrophies and desmin-related myopathies. To further our understanding of syncoilin function in skeletal muscle, we have a) analysed syncoilin expression in various mouse mutants, b) investigated post-translational modification and c) utilized several cell-lines to analyse filament formation.

The Assembly of Muscle Sarcoglycans in Cell Cultures. *W. Shi¹, J. Shottenfeld¹, L.M. Kunkel², Y. Chan¹.* 1) The Henry Hood Research Program, Sigfried and Janet Weis Center for Research, The Geisinger Clinic, Danville, PA; 2) Howard Hughes Medical Institute and Division of Genetics, Children's Hospital and Harvard Medical School, Boston, MA.

Sarcoglycans (SGs) are a group of four transmembrane proteins that form a complex on the muscle membrane. Mutations in SGs have been shown to cause the autosomal recessive form of Muscular Dystrophy known as Limb-Girdle Muscular Dystrophy. Mutation in one sarcoglycan often results in the loss of other sarcoglycans on the membrane. Using a heterologous expression system, we study the assembly and transport of the sarcoglycans in mammalian cell culture. Expression of all four sarcoglycans showed they are localized to the cell membrane. However, expression of the a-SG alone produced a Golgi-like immunofluorescent staining while b-SG, g-SG or d-SG each generated an ER-like pattern of staining. In addition, the staining pattern of b-SG revealed a uniform distribution in the ER by confocal microscopy while d-SG and g-SG often formed distinctive aggregates. Co-transfection of b-SG with other sarcoglycans demonstrated that 1) b-SG co-localized with either g-SG or d-SG to produce a uniform staining in the ER, 2) b-SG did not co-localize with a-SG in the absence of the other two sarcoglycans, 3) expression of both b-SG and d-SG is sufficient to cause their localization to the cell membrane. Finally, by mutagenesis, we have mapped the glycosylation and intra-molecular di-sulfide bond sites in the sarcoglycans. The results suggest that b-SG and d-SG / g-SG form a critical core for the assembly of the sarcoglycan complex during the early stage of their biosynthesis and they do not interact with g-SG until reaching the Golgi. Thus, there is a hierarchy in the assembly of sarcoglycans and their transport to the membrane is tightly controlled at each stage.

3D structure Modeling for human iduronate and Prediction of Effects by the mutation which was found in Korean patients with Hunter syndrome. *K.B. Moon¹, C.H. Kim², H.Z. Hwang², K.H. Paik¹, J.H. Yoon³, C.K. Han³, D. Jin^{1,2}.* 1) Pediatrics, Samsung Medical Center, Seoul, Seoul, South Korea; 2) Samsung Biomedical Research Center Dept. Medical Genetics; 3) IDR Tech Inc.

Hunter syndrome is a rare, X-linked, recessively inherited disease affecting multi-organ systems. The disease is caused by the inability to degrade dermatan sulphate and heparan sulphate, which is due to mutations in the iduronate-2-sulphatase gene (IDS). The mutations causing the disorder are heterogeneous, ranging from small micro-lesions to gross deletions and inversions. As the first attempt to examine the molecular changes of IDS gene in the Korean Hunter patients, we have screened DNA samples from 35 Korean families with Hunter syndrome using the polymerase chain reaction and single-strand conformation polymorphism analysis. As a result, we have identified and characterized 21 mutations (Table 1). Also 3D modeling structure of iduronate 2 sulfatase was constructed from the known X-ray structures of several sulfatases. We depicted the mutation data at the 3D structure of the enzyme to understand the importance of the mutation in term of functional significance. Our findings indicate that most of the mutation points of IDS gene is located at peripheral areas, but structurally critical points that can cause problem during refolding of protein, although the the mutations of IDS gene in the Korean Hunter syndrome patients are likely to be heterogeneous. Our findings consequently aid to understand the underlying mechanisms associated with this disease.

Modeling splice site and transcription factor binding site variation by information theory. *P.K. Rogan¹, S.R. Svojanovsky¹, I. Hurwitz¹, T.D. Schneider², J.S. Leeder¹*. 1) Children's Mercy Hosp, Kansas City, MO; 2) National Cancer Institute, Frederick, MD.

We have validated information theory-based models for human acceptor and donor splice sites and NF- κ B heterodimer binding sites. The average information describes the range of variation in sites having a common function, whereas the information content of a single site (R_i) measures its conservation within a family of binding sites. The strengths of different sites can be directly compared based on their respective R_i values, since R_i is related to the free energy of binding. The splice site models comprise a set of automatically curated donor ($n=111,772$) and acceptor ($n=108,079$) sites from all known genes in the human genome draft sequence. These comprehensive models accurately predict the effects of mutations, polymorphisms and cryptic splicing, including variants which partially abolish splicing and often produce milder clinical phenotypes. The NF- κ B model was derived initially from previously known strong sites and then iteratively refined by incorporating binding sites predicted from the initial model and validated by EMSA studies. The NF- κ B model accurately rank orders the strengths of known binding sites in competitor EMSA assays, and distinguishes promoters of genes regulated by NF- κ B from those in which transcription is not known to be induced. The model was validated by detecting known (and previously unrecognized) sites in promoters of each of 13 genes regulated by NF- κ B that were excluded from the initial model. The most sensitive and specific information theory-based models are based on sites spanning a wide range of binding affinities. A CCAAT-box protein binding site model ($n=175$) based on the TRANSFAC database accurately predicted a ³1.4 fold increase in binding strength due to a G>A substitution in the promoter of the A_γ -globin gene that results in HPFH. Many other transcription factor binding sites collated in TRANSFAC are biased towards strong binding sites. More representative models will be required to detect weaker binding sites and to reliably assess the effects of mutations. Supported by PHS R01 ES10885-02 and the Merck Genome Research Foundation.

Identification of a novel deletion mutation in the 3' untranslated region (3'UTR) of the lipoprotein lipase gene in Hispanics and its association with the components of glucose homeostasis. *H. Razzaghi*¹, *R.M. Hamman*², *M.I. Kamboh*¹. 1) Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA; 2) Dept Preventive Medicine and Biometrics, Univ Colorado, Denver, CO.

The enzyme lipoprotein lipase (LPL) plays a central role in lipid metabolism and many diseases, including atherosclerosis, obesity, and insulin resistant syndrome, appear to be directly or indirectly related to abnormalities in LPL function. Numerous studies have shown that the LPL gene is sensitive to hormonal regulation of insulin. However, the part of the gene that is insulin sensitive (insulin response element, IRE) has yet to be identified. Using bioinformatic techniques, we identified a 19 bp sequence as a putative IRE in the LPL 3'UTR (exon 10). We then targeted this region for mutational screening using SSCP in three population groups, including Hispanics (N=406), Whites (N=614), and Blacks (N=100). In the putative IRE, a novel five-base deletion mutation (5D2014) was identified only in Hispanics with a carrier frequency of 4.2%. Genotype-phenotype association studies of this ethnic-specific mutation with components of lipid and glucose homeostasis revealed its significant association with elevated insulin, C-peptide, and decreased fasting glucose. Analysis of the mRNA secondary structure of this mutation suggests that mutant RNA is less stable than the wild type. DNA-protein interaction studies using electrophoretic mobility shift assay (EMSA) of the putative IRE revealed binding sites for regulatory factor(s) from muscle cell nuclear extracts. Expression studies are in progress to test the involvement of the 5D2014 mutation in the LPL mRNA stability. In summary, we have identified a novel 5 bp deletion mutation in the putative IRE of LPL that alters the secondary structure of LPL mRNA and is associated with components of glucose homeostasis.

Analysis of the RNA component of human telomerase and its associated activity. *M. Dickman*¹, *J.P. Rose*², *M.A. Marino*³, *D.P. Hornby*². 1) Transgenomic Research Lab, Univ Sheffield, Sheffield, UK; 2) Department of Molecular Biology and Biotechnology, Krebs Institute, Univ Sheffield, Sheffield, UK; 3) Transgenomic, Inc., Gaithersburg, MD.

Functional RNA molecules possess a high degree of secondary and tertiary structure and often adopt complex conformations. Analysis of RNA *in vitro*, involves transcription, traditionally followed by purification and analysis by denaturing gel electrophoresis. The RNA generated in this way often appears as multiple species following electrophoresis. Here we report the use of ion pair reverse phase liquid chromatography under denaturing conditions using the WAVE® DNA Fragment Analysis System (Transgenomic, Inc.) to analyse the RNA component of human telomerase RNA. Furthermore, the chromatography separation process has been extended to analyse folded (or multimeric) RNA species by the incorporation of Mg²⁺ ions into the chromatography buffers. Conformational influences on the separation of human telomerase RNA in the presence of Mg²⁺ by chromatography is compared to gel electrophoresis of the same species. This novel chromatographic procedure permits analysis of the temperature dependent formation of folded (or multimeric) RNA species. We also demonstrate the ability to detect telomerase activity in a high throughput manner using a fluorescent based TRAP assay.

Identification of Cell Senescence Genes at 6q22-q23 and 6q26-q27 by Functional Analysis. *J. Liu^{1,2}, A.K. Sandhu², N. Rane^{1,2}, H.S. Sekhon³, G.P. Kaur², R.S. Athwal^{1,2}.* 1) Dept. of Pathology, School of Medicine, Temple University, Philadelphia, PA 19140, U.S.A; 2) Fels Institute for Cancer Research and Molecular Biology, School of Medicine, Temple University, Philadelphia, PA 19140, U.S.A; 3) Dept. of Biology, University of District of Columbia, Washington D.C.2008, U.S.A.

Applying a functional approach, we have identified two loci for replicative senescence on human chromosome 6q. The introduction of chromosome 6 or 6q restores normal cell growth and senescence in SV40 immortalized mouse and human cells (Sandhu, et al.1994 PNAS 91, 5498). Independent immortal revertant clones, originating from senescent microcell hybrid clones, containing human chromosome 6 or 6q, were analyzed for the presence or loss of chromosome 6 specific DNA markers. These data identified deletions of markers located at 6q22-23 and 6q26-27, suggesting the presence of two candidate growth suppressor loci on 6q. YAC and BAC clones, identified by the markers located in the candidate regions, were assembled to construct a high resolution physical and genetic map of the region. Computer analysis of the draft DNA sequence has identified several ESTs and cDNA mapped at 6q22-23 and 6q26-27. RT-PCR and Northern blot analysis of immortal cell lines, derived from SV40 transformed cells and tumors, for the expression of candidate cDNAs, revealed loss of expression for two cDNAs in 7 cell lines. Full-length cDNAs corresponding to candidate partial cDNAs have been cloned into an inducible mammalian cell expression vector. These cDNAs are currently being tested for the restoration of senescence and growth suppression in immortal cell lines, High incidence of LOH at 6q22-23 and 6q26-27, in multiple studies, suggest a role for these region in the etiology cell transformation and tumorigenesis..

Downstream of an RNA regulatory protein causing a common human disease: Expression profiling human SMN-deficient muscle. *C. Brandoli*¹, *S. Servadei*², *E. Hoffman*¹. 1) Res Ctr Genetic Medicine, Children's Natl Medical Ctr, Washington, DC; 2) Department of Neurology , Catholic University, Rome, Italy.

Spinal Muscular Atrophy (SMA type 1) is an autosomal recessive neuromuscular disease occurring 1 in 10,000 newborns. While the primary pathological feature of SMA has long been considered to be the loss of the cell bodies of alpha-motor neurons in the anterior horns of the spinal cord, increasing evidence points to important defects of the end target of the motor neurons, namely skeletal muscle. SMA is caused by reduction of the expression of the survival of motor neuron (SMN) gene, which is thought to regulate a series of downstream mRNAs, and the protein is highly concentrated at the neuromuscular junction. Downstream targets have not yet been defined. We describe the use of gene expression profiling of muscle biopsies from human SMA type I infants and age/sex-matched controls to identify genes that were potential downstream targets of SMN mRNA regulation. We found specific transcription factors to be highly down-regulated relative to normal age-matched controls, and disease controls (neonatal Duchenne muscular dystrophy patients). Most striking was a 24-fold under expression of a specific Zinc finger transcription factor of otherwise unknown function. These genes become potential down-stream targets of SMN regulation in muscle.

The genetic variation of the small mucin gene MUC7. *K. Rousseau¹, L. Vinal¹, J.A. Holloway², D.M. Swallow¹.* 1) The Galton laboratory, Biology, University College London, London, UK; 2) Human Genetics Division, University of Southampton, Southampton General Hospital, Southampton, UK.

Background: The mucins are a large heterogeneous group of glycoproteins, which share the common feature of having a central domain of tandemly repeated sequence (TR). This domain often shows VNTR type polymorphism and carries most of the glycosylation. MUC7 is a small mucin (377 amino acid residues) which is an important component of the saliva, a major defence barrier against the environment. This mucin has been shown to have anti-fungal activity and interacts with bacteria. Previous studies in our laboratory have indicated that MUC7 plays a role in predisposition to asthma and possibly other chest diseases. There are two common MUC7 TR alleles, one with 5 repeats and the other with 6. The 5 repeat allele is under-represented in patients with asthma. Although the size difference, 23 amino acids, would also alter the number of carbohydrate side groups, this small relative reduction in size seems unlikely to be responsible for the protective effect. Thus it is our hypothesis that other associated single nucleotide polymorphisms (SNPs) are responsible for the association. **Aims:** The aim of this project is to screen MUC7 for polymorphism in non-TR regions to define haplotypes in order to study patterns of association with chest diseases. **Methods:** A combination of bioinformatics and experimental tools were used. Published sequences and those available from the human genome sequencing project were aligned. SNP data were collected and the regions likely to play a role in regulation of expression were then screened using denaturing High Performance Liquid Chromatography (dHPLC) and sequencing. **Results and Conclusions:** The SNP information in the databases was relatively poor with no meaningful allele frequencies being given. However some were confirmed and we have also identified SNPs that had not been reported before. Some, particularly those in the promoter region and the first intron, could be functionally significant and are being investigated further. The haplotypes and allele frequencies are now being studied in patients suffering from chest diseases.

Characterization of regulatory networks controlling Ectodysplasin-A expression: progress toward identification of enhancer-binding proteins. *M.C. Durmowicz, C.-Y. Cui, D. Schlessinger.* Laboratory of Genetics, National Institute on Aging, Baltimore, MD.

When mutated, the *EDA* gene causes the X-linked recessive disorder anhidrotic ectodermal dysplasia, which is characterized by the defective development of skin appendages such as hair follicles, teeth, and sweat glands. We have begun to characterize the function of the *EDA* gene in ectodermal development by studying the regulatory networks controlling *EDA* gene expression. Our previous work has identified multiple regulatory regions within the *EDA* promoter, each of which is responsible for successively more specific levels of control. Here, we focus on the 123-bp enhancer region identified by transfection studies and electrophoretic mobility shift assays. Within this area, two 40-bp regions were shown to be necessary for enhancer function by transfection of deletion constructs of the *EDA* promoter region. Computer analysis identified three potential binding sites for transcription factors; one each for members of the GATA and Nkx families of transcription factors, involved in cardiac development, and one for an unknown binding protein that is found in an *EGFR* enhancer. In this work, two of the enhancer-binding sites, which include the computer identified Nkx and EGFR-related sites, have been experimentally confirmed by gel shift assays. Southwestern blotting of nuclear extract from HeLa cells shows at least two major species, of approximately 125 kD and 60 kD, that bind to both critical enhancer regions. In an effort to isolate and identify these species, HeLa nuclear extract was fractionated with salt gradient elution on a Heparin column. One fraction has been identified that exhibits binding to the region containing the putative GATA and Nkx sites in both gel shift assays and Southwestern blotting. Several fractions also exhibit binding to the EGFR-related site in gel shift assays. Identification of these enhancer-binding species by mass spectrometry is currently underway.

Generation of a genome-wide library of regulatory sequences through the use of a novel DNase hypersensitive site cloning procedure. *G.E. Crawford, I. Holt, C.M. Robbins, T. Moses, T.G. Wolfsberg, F.S. Collins.* NHGRI, NIH, Bethesda, MD.

Initial sequence analysis of the human genome has identified approximately 30,000-40,000 genes, but relatively little is known about how most of these genes are regulated. DNA binding proteins such as transcription factors, repressors, and insulators typically displace histones, making their presence detectable by DNase hypersensitivity (HS) and footprinting assays. Many *in vivo* DNA binding sites for specific genes have been identified to date, but current experimental procedures are limited by size constraints (<20 kb) and the number of genes that can be studied at one time. We have designed a protocol to generate a genome-wide library of regulatory sequences by cloning DNase hypersensitive sites from human K562 erythroleukemia cells, a line in which the DNase hypersensitivity of the globin loci has been extensively characterized. Genomic DNA from intact nuclei was digested with DNase at concentrations optimized for the beta-globin locus control region, polished to generate blunt ends, digested to completion with common restriction enzymes, cloned, and sequenced. The DNase treated library contained approximately 400,000 clones, a number ten fold higher than that of the no DNase control, indicating that very few clones resulted from random shearing. Initial analysis of several hundred clones revealed that approximately 30% of these clones mapped to within 10 kb of a known gene. Of the sequences contained within genes, >50% mapped within the first or second intron, consistent with what is currently known about the location of intergenic regulatory sites. These preliminary data indicate that our library appears to be enriched for regulatory elements involved in the control of gene expression. Deeper library sequencing should identify valid regulatory elements by repeated observation of the same sequence. This procedure, which can be applied to any cell line or tissue, will be useful in identifying regulatory elements controlling global expression differences that delineate tissue types, stages of development, and disease susceptibility.

Tissue specific expression of the branched-chain a-ketoacid dehydrogenase kinase. *E.A. Muller¹, D.J. Danner²*. 1) Genetics and Molecular Biology, Graduate Division of Biological and Biomedical Sciences, Emory University, Atlanta, GA; 2) Department of Human Genetics, Emory University School of Medicine, Atlanta, GA.

The branched-chain a-ketoacids from leucine, isoleucine and valine are oxidatively decarboxylated by the branched-chain a-ketoacid dehydrogenase complex (BCKD). BCKD is present in the mitochondria of every tissue, and its catabolic activity is regulated primarily through changes in the phosphorylation state of the complex. A specific kinase (BCKD kinase) is differentially expressed in tissues, and functions to add the inactivating phosphate. The mechanism for tissue specific expression of BCKD kinase is not known. Northern blot analysis indicates that murine BCKD kinase mRNA is present at a low level in the liver, and in contrast, is highly expressed in skeletal muscle. This pattern is paralleled for the mouse BCKD kinase protein. To address whether mRNA differences among tissues are a result of transcriptional or post-transcriptional regulation, we performed transient transfection studies with luciferase reporter constructs bearing the entire 5' UTR of the mouse BCKD kinase gene and increasing amounts of 5' DNA sequence. BNL Cl.2 liver cells and differentiated C2C12 myotube cell lines modeled mouse liver and skeletal muscle, respectively. Reporter gene expression was higher in the myotubes than in the hepatocytes, depending on the amount of putative promoter sequence used. DNase I protection experiments and gel mobility shift assays are being used in conjunction with chromatin structure assessments to further define the regions in DNA responsible for the tissue specific differences in expression levels of BCKD kinase.

Gene expression profiling endometriosis in Finland. *M. Mahadevappa¹, C. Liu¹, S. Oksjoki², M. Setälä², L. Anttila², E. Vuorio², J.A. Warrington¹.* 1) Health Management Research, Affymetrix, Santa Clara, CA; 2) Dept. of Medical Biochemistry and Molecular Biology, Kiinamyllynkatu, University of Turku, Kiinamyllynkatu 10, Turku, Finland 20520.

Ten to fifteen percent of all women in their reproductive years are diagnosed with endometriosis. The pain caused by endometriosis can be debilitating and the disease often results in infertility. In endometriosis, abnormal endometrial lesions grow within the uterus as well as outside of the uterus including the ovaries, bowel, colon and rectum. Like normal endometrial tissue, these endometrial lesions and endometriomas respond to hormonal changes occurring during the menstrual cycle by proliferating and secreting. Currently the molecular mechanism of endometriosis is not well understood and there is no cure. In an attempt to understand the etiology of endometriosis, gene expression profiling was carried out on samples from patients in Finland. Finland has a well-organized medical system. Many endometriosis patients have a laproscopic exam prior to hormonal or medical intervention. In our study, all patients were diagnosed by the same clinical staff and all samples were freshly collected during laproscopic exam at diagnosis prior to hormonal treatment. Gene expression monitoring was carried out on 18 normal uterine samples, 12 peritoneal lesions of the uterus and 15 ovarian endometriomas. Targets were hybridized to high density oligonucleotide arrays containing probes for approximately 22,000 genes. Candidate genes are identified by multiple methods including both non-parametric, Mann-Whitney, and parametric, Students T, tests. Hierarchical clustering using candidate genes accurately segregates different tissue types. A subset of tens of candidates were selected for confirmation by QRT-PCR and those results will be presented. All candidate genes are annotated with locus and functional category information. Genes in pathways of particular interest will be discussed.

Sp1 CAN INCREASE *CFTR* PROMOTER ACTIVITY. *P.J. Mogayzel Jr, T.L. Wagner, P.L. Zeitlin.* Department of Pediatrics, The Johns Hopkins School of Medicine, Baltimore, MD.

Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the cystic fibrosis transmembrane conductance regulator gene (*CFTR*). *CFTR* is expressed at low levels in specialized epithelial cells in several tissues. The *cis*-acting elements that regulate *CFTR* expression are not well characterized in part because of this low level expression. Only a few DNA elements within the minimal promoter have been confirmed to influence *CFTR* expression. Although there is no TATA box the *CFTR* promoter has several consensus sequences for transcription factor binding, including 3 Sp-1 sites located at -1022, -405 and -326 bp upstream from the translational start site. We investigated if binding of Sp1 to these sites could influence *CFTR* promoter activity. The function of these Sp1 consensus regions was assayed by co-transfecting *CFTR* promoter-containing plasmids with an Sp1 expression vector. We constructed plasmids in which 95, 118, 262, 354 or 1993 bp of *CFTR* 5'-flanking DNA were inserted upstream of the luciferase reporter gene in pGL3-basic (Promega, Madison, WI). These *CFTR* promoter-containing plasmids were then transfected into an immortalized fetal human tracheal epithelial (FHTE) cells or human bronchial epithelial cells derived from a CF patient (IB3) cell in the presence or absence of a plasmid that expressed Sp1 driven by the CMV promoter. Transfection efficiency was assessed by co-transfection of a *Renilla reniformis* luciferase-containing plasmid (pRL-TK, Promega). The presence of Sp1 increased *CFTR* promoter activity of the 354 and 1993 bp fragments by 2-4 fold in both cell lines. Sp1 increased *CFTR* promoter activity of the 262 bp fragment by 0.5-1 fold. These data suggest that Sp1 can increase *CFTR* promoter activity and may play a role in the regulation of *CFTR* expression. This work was supported by the Cystic Fibrosis Foundation.

Analysis of the *RUNX2/CBFA1* promoter in cleidocranial dysplasia identifies cis elements that regulate the *CBFA1* transcriptional network. *D. Napierala, R. Mendoza, Q. Zheng, G. Zhou, E. Munivez, Y. Chen, B. Lee.*
Molecular and Human Genetics, Baylor College Medicine, Houston, TX.

Cbfa1 is one of three mammalian orthologs of the *Drosophila runt* gene and encodes a transcription factor essential for osteoblasts differentiation. We and others have shown that *CBFA1* mutations cause cleidocranial dysplasia (CCD), a dominantly inherited skeletal dysplasia characterized by delayed endochondral and intramembranous ossification. The majority of mutations are missense and abolish *CBFA1* binding to a target OSE2 sequence. These and additional nonsense, splicing and frame shift mutations are detectable in approximately 60% of CCD patients. Based on transfection and DNA binding studies, the CCD phenotype arises from haploinsufficiency. To identify additional determinants of the *CBFA1* transcriptional network, we performed sequence analysis of the 1.4 kb proximal *CBFA1* promoter and of the *CBFB* gene in 27 CCD patients without mutations in the *CBFA1* coding region. Mouse studies have shown that the corresponding mouse *Cbfa1* promoter is sufficient for high level expression in osteoblast cell lines and that *CBFB* is an interacting protein critical for RUNT domain function. While no pathogenic mutations of *CBFB* were found in our cohort, a total of 4 sequence variants in the *CBFA1* promoter was detected. DHPLC analysis in 180 control chromosomes showed that an A>C substitution found in two CCD patients, and a G>T substitution found in four CCD patients have allele frequencies of 10% and 15%, respectively, in the general population. We did not observe an increased frequency of these SNPs in a cohort of women with premenopausal osteoporosis. Two other sequence variants were found unique to the CCD group. Both occur within zinc finger transcription factors binding sites. Electrophoretic mobility shift assay (EMSA) using nuclear extracts from ROS17, 10T1/2 and MCT cells showed a specific protein-DNA complex corresponding to each cis element. The analyzed *CBFA1* promoter variants exhibited different DNA-protein binding specificity. Thus, the allelic spectrum of CCD may help us identify important cis-elements that bind trans-acting factors that regulate the *CBFA1* expression.

Activation of the human hepatic lipase gene promoter in vitro by a combination of HNF4 and Coup-TFII/ARP-1 and in vivo by 9-cis retinoic acid. *B. Kurdi-Haidar, S.S. Deeb.* Departments of Medicine and Genome Sciences, Univ Washington, Seattle, WA.

High levels of plasma hepatic lipase (HL) activity are correlated with an atherogenic lipoprotein profile characterized by decreased high-density lipoprotein and prevalence of small, dense low-density lipoprotein. Therefore, modulation of its activity may alter the risk for development of atherosclerosis. The proximal promoter of the HL gene contains 2 direct repeats (DR) of the core motif 5-PuG[G/T][T/A]CA-3, a DR1 and a DR4, that typically bind retinoic acid, vitamin D, and steroid/thyroid hormone nuclear receptors. We investigated the transcription factors that modulate activity of the HL promoter through interaction with these two DRs. First, we conducted functional analysis of the wild type and mutant HL proximal promoters driving expression of the luciferase reporter gene by transient transfection of COS7 cells. Cotransfection with the wild type HL promoter and plasmids expressing the hepatocyte nuclear factor 4 (HNF4) and the chicken ovalbumin upstream promoter transcription factor II (Coup-TFII)/apolipoprotein regulatory protein 1 (ARP-1) resulted in five-fold activation. Analysis using reporter constructs with mutations at the DR1 or DR4 sites indicated that the DR1 element is necessary for activation by the HNF4-Coup-TFII/ARP-1 combination. Second, we determined the effect of treatment with ligands for the nuclear receptors RXR and LXR on endogenous HL mRNA levels in the human hepatoma cell line HuH7 by quantitative real-time RT-PCR. While the ligand of the nuclear receptor RXR, 9-cis retinoic acid resulted in 2-fold increase in HL mRNA, the LXR agonist T0901317 had no effect. In conclusion, the HL promoter is activated by the HNF4-Coup-TFII/ARP-1 combination using the DR1 site, and RXR but not LXR may be involved in its in vivo activation.

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Isolation, characterization and analysis of the ALX4 promotor region. *W. Wuyts, P. Verdyck, W. Van Hul.* Medical Genetics, University of Antwerp, Antwerp, Belgium.

The Alx4 gene is a homeodomain transcription factor related to the *Drosophila aristaless* gene which plays an important role in craniofacial and limb development. This is demonstrated by Alx4 KO mouse showing preaxial polydactyly, ventral body wall defects and a delay in the formation of the parietal bone of the skull. Previously, we isolated the human ALX4 located on chromosome 11 in a region which is deleted in patients suffering from the Proximal 11p Deletion Syndrome (P11pDS) and we showed that in humans inactivating mutations in ALX4 cause skull ossification defects (Foramina Parietalia Per magna; FPP). Starting from BAC and PAC clones the 5' region flanking the ALX4 gene of both human and rat was isolated and characterized. Reverse transcriptase and RACE experiments were performed and enabled us to map the ALX4 transcription start site (TSS) approximately 700 bp preceding the ATG startcodon. Alignment of human and rat promotor sequences and computeranalysis identified several potential regulatory regions. Deletion constructs surrounding the TSS were generated by PCR and exonuclease/S1 treatment and analyzed by a dual Luciferase reporter assay for their promotor activity in osteoblastic cell lines. Regions associated with a significant increase or decrease in promotoractivity were further characterized to identify and verify potentially binding sites for known transcriptionfactors. This enabled us to identify several potential regulators of ALX4 expression which are currently being investigated.

PITX2 and MEF2A synergistically activate the ANF promoter. *I. Saadi*¹, *A. Kuburas*², *A.F. Russo*^{1,2}. 1) Genetics Program,; 2) Department of Physiology and Biophysics, University of Iowa, Iowa City, IA.

PITX2 is a bicoid-type homeodomain protein that is mutated in patients with Axenfeld-Rieger syndrome (ARS), and is required for development of the heart. PITX2 knock-out mice show defects in cardiac positioning and atrial septation. In order to determine the role of PITX2 in the heart, we looked at atrial natriuretic factor (ANF) and myocyte enhancing factors (MEF2) which are known regulators of cardiac development. ANF is one of the atrial natriuretic polypeptides that are involved in electrolyte homeostasis in the cardiovascular system. MEF2A is highly expressed in skeletal muscle, cardiac muscle and the brain. In addition, MEF2C knockout mice show a complete abolition of ANF expression. Our studies showed that PITX2 can activate the ANF promoter in LS8 (dental epithelium) and CHO (chinese hamster ovary) cell lines. Interestingly, cotransfection of MEF2A and PITX2 synergistically activated the ANF promoter in LS8 and CHO cells. This synergism was not observed with the synthetic TK-Bicoid reporter or the GAD1 promoter. The synergism requires the homeodomain of PITX2. At least two of the ARS mutations that abolish DNA binding of PITX2 could not synergize with MEF2A. We also did transfections in the presence of MKK6, a p38 kinase activating kinase known to activate MEF2A. MKK6 cotransfection with either PITX2 isoform a or c significantly increased the fold activation of the ANF promoter in LS8 cells. This increase was also seen in the presence of MEF2A. However, the synergism of MEF2A with PITX2c was significantly greater than that with PITX2a. Yeast two-hybrid analysis shows that PITX2a does interact with MEF2A. The interaction requires the N-terminus and homeodomain of PITX2a. We are in the process of determining whether PITX2c and MEF2A show a stronger physical interaction. These studies indicate that the PITX2-MEF2A synergism may coordinate ANF peptide expression during cardiac development.

Study of ABCG5 and ABCG8 Genes Regulation. *S. Shulenin, T. Annilo, M. Dean.* LGD, NCI-Frederick, Frederick, MD.

Two new human ATP-binding cassette transporter (ABC transporter) genes (ABCG5 and ABCG8) were found and characterized. The primary structure of these genes shows high homology to the ABCG (White) subfamily of ABC transporters, that includes half-transporter proteins. Previously we have shown that ABCG5 and ABCG8 are involved in cholesterol and sterol transport and cause the rare human disease sitosterolemia. That transport processes that regulate sterol homeostasis in the human body are poorly understood and the study of the regulation of these genes could help to better understand this process.

An initial study of the expression of these genes in different tissues and cells shows independent regulation of these genes. The genes are located on chromosome 2p21 head-to-head to each other and the promoter area between the ATG codons is about 300 base pairs. To understand mechanism of ABCG5 and ABCG8 regulation and identification of the factors that are involved in gene regulation we analyzed DNA the sequence of these genes from different species and applied a gel-shift analysis in order to identify regulatory factors. Comparative analysis of homologous genes from different species revealed few conservative sequences in the promoter area and introns that could serve as binding areas for regulatory elements. Synthetic oligonucleotides that correspond to these sequences were used for gel-shift experiments. These experiments have shown that two proteins bind in the promoter and intronic regions of these genes. DNA fragments that were positive in gel-shift experiments were used for the purification of binding proteins. Identification of proteins that bind to DNA will help to understand their involvement in ABCG5 and ABCG8 genes regulation and mechanism of sterol homeostasis.

Heterogeneous Allele Expression Of Pulmonary Surfactant Protein (SP)-D Gene In Rat Large Intestine And Other Tissues. *Z. Lin¹, J. Floros^{1, 2}*. 1) Dept Cell Mol Physiol, Penn State Univ college Med , Hershey, PA; 2) Dept Pediatrics, Penn State Univ college Med , Hershey, PA.

Epigenetic regulation plays important roles in gene expression. Random allele expression has recently been observed for several genes including interleukins and genes of the lymphoid system. We studied the hypothesis that pulmonary surfactant protein (SP)-D, an innate host defense molecule, exhibits random allele expression in a tissue-specific manner. SP-D gene expression is tissue specific in the 14 tissues studied. SP-D is expressed in lung, large and small intestine, stomach, ear, eye, liver, vagina, and penis, but it is not expressed in tongue, heart, brain, kidney, and spleen as assessed by either Northern or RT-PCR. Study of SP-D allelic expression in several tissues revealed a balanced biallelic (BB) in lung, and, in several extrapulmonary tissues, a heterogeneous pattern: BB, imbalanced biallelic (IB), and monoallelic (MO). The results from 103 heterozygous rats showed an expression profile in large intestine of BB (22%), IB (60%), and MO (18%). Among 8 families, the percent of BB in siblings varied from 0 to 38%, and MO from 0 to 38% and approximately half of the siblings had IB. Allele T is more frequently found in monoallelic expression than allele C (MOT (16%) and MOC (2%)). The parent-of-origin does not play a role in SP-D allele specific expression. However, acquired epigenetic factors, family background or other may contribute to the overall pattern of expression. Allele expression differences point to yet another level of genetic variability where deranged allele expression may contribute to disease susceptibility through phenotypic differences in the expression level and/or the loss of the biallelic expression advantage. The significance of the monoallelic and/or the heterogeneous allele expression of SP-D under normal or adverse conditions is unknown and subject to speculation. This work is supported by NIH R37HL34788 and AHA198312P.

Identification of different elements within exonic splicing enhancer sequence of the dystrophin gene. A. Surono¹, Y. Takeshima², M. Matsuo¹. 1) Div of Molecular Medicine, Kobe University Graduate School of Medicine, Kobe, Japan; 2) Dept. of Pediatrics, Kobe University Graduate School of Medicine, Kobe, Japan.

Exonic splicing enhancers (ESEs) are RNA sequences required for accurate splice site recognition and the control of alternative splicing by recruitment general splicing factors. Previously, we demonstrated that a 31-nt element of exon 19 of the dystrophin gene (named as element A) functions as a *cis*-acting element for the correct maturation of dystrophin mRNA. In order to characterize element A, we separated element A into three overlapped fragments, the 5' region (element A1), the middle region (element A2) and the 3' region (element A3). Each fragment was analyzed for nuclear protein binding ability and enhancer activity by *in vitro* and *in vivo* splicing assay.

RNA binding protein was analyzed by UV crosslinking and mobility shift assay using fluorescence labeled RNA probes of each element and HeLa nuclear extract (HNE). We demonstrated that element A1 bound specifically to nuclear protein of 130-kDa. However, neither element A2 nor A3 has protein-binding site. *In vitro* splicing assay using *chimeric dsx* pre-mRNA revealed that element A1 was able to enhance splicing of the upstream intron as equal as element A, but not for elements A2 and A3. In addition, mutational analysis using dystrophin minigene expressed in HeLa cells demonstrated that when either element A1 or A2 was deleted, exon 19 was skipped during maturation of dystrophin mRNA. In contrast, deleted element A3 did not affect exon 19 splicing. These data indicate that elements A1 and A2 activate exon 19 incorporation into mRNA in different manner.

This is the first evidence demonstrating different character within ESE.

Identification of alternative splicing variants of human biotinidase and their possible role in the intracellular localization of the enzyme. *C.M. Stanley¹, A.J. Hymes¹, G.C. VanTuyle¹, J.W. Bigbee¹, B.T. Webb¹, B. Wolf².* 1) Medical College of Virginia of Virginia Commonwealth University, Richmond; 2) Connecticut Children's Medical Center and the University of Connecticut School of Medicine, Hartford.

Biotinidase is essential for recycling the vitamin biotin. Biotinidase deficiency is an inherited disorder characterized by neurological and cutaneous symptoms that can be successfully treated or prevented with biotin. Biotinidase has at least two enzymatic functions that may require it to be localized to various cellular and/or extracellular sites. RACE-PCR was used to extend the 5' sequence of exon 1 by 90 bases and identify three novel, alternatively spliced variants of biotinidase, 1a, 1b and 1c, in multiple human tissues. Exon 1c was present only in testes and includes sequence originally designated as intron 1. Intron 1 sequence was identified in GenBank (AC027129) and completes the 5' structure of the gene. The 5' splice variants, 1a and 1b, contain sequence motifs that suggest biotinidase localizes to the mitochondria/nucleus and endoplasmic reticulum, respectively. Immunohistochemical and organelle isolation studies were used to determine the intracellular localization of biotinidase. Immunohistochemical studies demonstrate that biotinidase localizes to the cytoplasm, but not the nucleus, of human fibroblasts and Hep G2 cells. Organelle separation by isopycnic gradient centrifugation of rat liver identified an 85 kDa biotinidase protein that had both biotinyl-hydrolase and transferase activities in fractions enriched for a microsomal marker enzyme. These results confirm earlier studies localizing biotinidase to microsomes. A 48 kDa protein that immunoreacted with anti-biotinidase was found in fractions enriched with a mitochondrial marker enzyme. The 48 kDa protein is biotinylated, is localized to the inner mitochondrial matrix, but has neither biotinyl-hydrolase nor transferase activities. The 5' splice variants and organelle fractionation studies suggest that biotinidase is directed to the secretory pathway and to mitochondria, but the function of the protein in mitochondria remains to be determined.

The splice of height: dual enhancers function in GH1 splicing. *R.C.C. Ryther¹, L. McGuinness², J.A. Phillips III³, C.T. Moseley³, C.B. Magoulas², I. Robinson², J.G. Patton¹.* 1) Biological Sciences, Vanderbilt University, Nashville, TN; 2) Molecular Endocrinology, Nat. Inst. for Medical Research, London, England; 3) Pediatrics, Vanderbilt University School of Medicine, Nashville, TN.

Isolated growth hormone deficiency type II (IGHD II) is characterized by short stature due to GH deficiency caused by dominant negative mutations in the GH1 gene. GH1 has five exons and four introns that are alternatively spliced to produce five known isoforms. These isoforms include all five exons (22 kDa), deletion of 15 codons of exon 3 (20 kDa), skipping of exon 3 (17.5 kDa), and deletions of exons 3-4 (11.3 kDa) or exons 2-4 (7.4 kDa). IGHD II mutations misregulate splicing of GH1 transcripts, leading to increases in the 17.5 kD isoform, which for unknown reasons has a dominant negative effect. To determine how GH1 splicing is regulated and why its perturbation causes IGHD II, we studied the role of exonic and intronic splicing enhancers (ESE and ISE, respectively) as well as a transgenic model of IGHD II. We focused on mutations that disrupt an ESE in exon 3 and an ISE in intron 3, causing aberrant splicing of exon 3 and abnormal increases in the 17.5 kDa isoform. The dual enhancers exhibit differential effects on the weak splice sites surrounding exon 3 but combine to ensure exon 3 definition. To determine the mechanism by which the 17.5 kDa isoform causes IGHD II, transgenic mice expressing the human 17.5 kDa isoform were created. Interestingly, these mice exhibit the dominant dwarfism phenotype of IGHD II with anterior pituitary hypoplasia, massive loss of somatotrophs, and gross secretory abnormalities in the few remaining somatotrophs. Our data show that IGHD II results from splicing mutations that disrupt recognition of exon 3, increasing the 17.5 kDa isoform which prevents maturation of GH dense secretory vesicles and causes subsequent cell death *in vivo*. Our results suggest that multiple exonic missense and silent mutations, as well as intronic variations, can perturb splicing enhancers leading to aberrant splicing and disease.

Alternative splicing: a key to dystrophin function. *M.M. Ferguson*^{1,2}, *P. Demacio*^{1,2}, *F. Cisternas*^{1,2}, *P.N. Ray*^{1,2}. 1) Department of Genetics, Hospital for Sick Children, Toronto, ON, Canada; 2) Department of Molecular and Medical Genetics, University of Toronto.

Duchenne muscular dystrophy (DMD) is a severe X-linked recessive disorder that is caused by mutations in the large and complex dystrophin gene. Although DMD is characterized primarily by progressive muscle wasting, patients commonly present with cardiac and respiratory complications. There are also several non-muscle phenotypes, including abnormal retinal neurotransmission.

Dystrophin is expressed as a family of structurally diverse protein isoforms that have different localizations and functions. Part of this diversity is due to alternative splicing at the 3' end of the transcript. The most common splicing event involves exon 78 and changes the C-terminus of the resultant protein from a hydrophilic C-terminus (DCT) to a hydrophobic C-terminus (ACT). In comparison to the DCT, the ACT is highly conserved across species and displays differential tissue expression and localization patterns. This suggests a unique functional role for this spliced region.

Immunofluorescent analysis was used to determine if the two different C-termini of dystrophin have different localization patterns in the heart and in the retina. In the heart, isoforms with the DCT are restricted at the plasma membrane surrounding the cardiomyocytes while isoforms with the ACT display an expression pattern along the transverse tubular system. In the retina, isoforms with the DCT show predominant staining at the outer plexiform layer while isoforms with the ACT show predominant staining at the inner limiting membrane and surrounding the microvasculature.

The high degree of sequence conservation and differing localization patterns suggest a unique function for the two C-termini of dystrophin. We hypothesize that these functional differences arise through differential protein interactions. To elucidate the functional significance of the splicing event involving exon 78 we have used library-based screening methods to identify proteins that interact specifically with the ACT.

Characterization of *Rai1* as a candidate gene for Smith-Magenis syndrome. *R.E. Lucas¹, C.N. Vlangos¹, S.H. Elsea^{1,2,3}.* 1) Genetics Graduate Program, Michigan State University, East Lansing, MI; 2) Department of Zoology, Michigan State University, East Lansing, MI; 3) Department of Pediatrics and Human Development, Michigan State University, East Lansing, MI.

Smith-Magenis syndrome (SMS) is a multiple congenital anomalies/mental retardation syndrome and possible contiguous gene deletion syndrome associated with an interstitial deletion of human chromosome 17p11.2. SMS has a recognizable behavioral and physical phenotype and occurs in approximately 1 in 25,000 births. We are interested in determining the molecular basis of SMS and have recently created a large-insert genomic clone contig of the SMS critical deletion region. Human *retinoic acid-induced 1 (RAI1)* is novel gene which codes for 1863 amino acids and maps to the central region of the SMS critical interval, adjacent to *SREBF1*. In order to assess *RAI1* as a candidate gene for SMS, we are currently analyzing the mouse homolog, *Rai1*, which maps to mouse chromosome 11 BACs RPCI-23 326M22 and 456O18 in a region of the mouse genome which is syntenic to the SMS critical interval. *Rai1* is 1840 amino acids and has approximately 77% overall identity to the human protein. *Rai1* is an attractive candidate gene for SMS because northern analysis demonstrates abundant mRNA expression in brain and database sequence analysis reveals regions of sequence homology with the transcription factor *TCF20*. In addition, based on the phenotypic analysis of patients who harbor deletions within the critical interval, *RAI1* maps to a genomic region which may contain a gene that has an effect on craniofacial development. Our laboratory is especially interested in transcription factors which may be dosage sensitive as well as the potent effects of retinoic acid on embryonic development. In order to determine a more precise expression pattern for *Rai1*, we are performing *in situ* hybridization on sectioned mouse embryos as well as constructing a knockout vector to assess the role of *Rai1 in vivo*.

Generation of Reporter Constructs for High Throughput Functional Analysis of Gene Variants. *R.H. Lipsky, F.-L. Lu, A.S. Card, A.J. Marko.* Dept Neurogenetics, NIAAA/NIH, Rockville, MD.

Single nucleotide polymorphisms (SNPs) in gene coding regions are rare but are likely to contribute significantly to changes in cell signaling pathways, resulting in long-term gene responses. To gain further understanding of variant protein function, it will be necessary to develop high throughput approaches for evaluating SNPs. We developed a qualitative and quantitative method for large-scale high throughput analysis of functional receptor variants. Two reporter gene fusion constructs were made. The first (pdsRed2.1GFP-1XCRE) contained a single copy of a human-derived cyclic AMP response element (CRE). The second (pdsRed2.1 GFP-1XNFkB) incorporated a human-derived NFkB binding site. Each sequence was fused to red-shifted Green Fluorescent Protein (Red GFP) using the plasmid pdsRed2.1. To validate functional activity of the NFkB reporter, we exposed NR2A cells transfected with pdsRed2.1 GFP-1XNFkB to Tumor Necrosis Factor- α (TNF- α), a known NFkB activator. Activation of the CRE reporter plasmid was confirmed by exposing NR2A cells carrying the pdsRed2.1GFP-1XCRE to forskolin, a known activator of CREB. Transfection efficiency was controlled by a reference plasmid that expressed GFP. Because Red GFP and GFP have distinct excitation and emission spectra, ratios of relative fluorescence (Red GFP/GFP) can provide quantitative gene expression data in response to different stimuli. Prior to quantitative analysis, we determined if TNF- α would activate the NFkB reporter construct. TNF- α (100 U/mL) activated NFkB-mediated gene expression, as shown by the presence of Red GFP in the transfected cells. The control cells, which did not receive TNF- α , did not exhibit Red GFP expression, showing that the NFkB reporter construct was not activated. Both control and experimental cells exhibited GFP, as expected. Similar results were obtained for the CRE reporter in the presence of forskolin (1-5 M). Our results show that our approach is sensitive and specific, and support the idea that dual fluorescent markers in real-time can be used to study responses to different stimuli, and to evaluate the role of functional SNPs in components of signal transduction pathways.

Structural analysis of a human nectin-like gene, fifth member of the nectin / PRR family of adhesion molecules.

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Objective: Identify, characterize, and perform mutational analysis on brain expressed genes in the 1q23 critical region of linkage for a large previously described hemiplegic migraine family. Background: Among the superfamily of immunoglobulin-like adhesion molecules there are four members of the nectin / poliovirus receptor related (PRR) genes coding for receptors of alpha herpes simplex family viruses expressed at intercellular junctions. Nectin 1 is thought to be involved in synapse formation, is found at high levels in sensory neurons of rat, and binds HSV glycoprotein D for virus entry into cells. We cloned and characterized a fifth member of the nectin/PRR family, most closely related to nectin 1 and nectin-2, between D1S2355 and D1S2635 at 1q23. Methods: Insilico database searches, analysis with programs such as BLOCKS, Compel, MatInspector, standard cloning, RT-PCR, and 5', 3' RACE were used. SSCP of 8 affected individuals selected from all family branches and 4 control individuals were used for mutational analysis of the gene including intron-exon boundaries. Results: The nectin-like gene codes for a transmembrane protein with three extracellular Ig-like domains (V and two C type) that share ~31% and 25.4% amino acid identity with Nectin 1 and 2, respectively. We found two alternative splice sites in the untranslated portion of an extended last exon in the human nectin-like gene. Analysis of the putative promoter region suggests predominantly brain expression, as confirmed by RT-PCR. The nectin like gene was found to be widely expressed, with alternatively spliced isoforms in brain, muscle, placenta, lung and lymphocytes. Only the brain specific isoform appears to skip exon 2, which encodes a unique region similar to the immediate early protein for herpes simplex virus (HSV). Nine single nucleotide polymorphisms were identified, none with complete segregation in hemiplegic migraine individuals. Conclusion: The brain specific isoform of this new member of the nectin / PRR family may have a role in cell-to-cell spread of HSV or other related viruses that should be further explored.

Genotype phenotype relation of TNF α as a risk marker for coronary atherosclerosis. *U. Schagdarsurengin¹, S. Schulz¹, T. Suess¹, U. Mueller-Werdan², K. Werdan², C. Glaeser¹.* 1) Inst. of Human Genet., MLU, Halle, Germany; 2) Dep. of Intern. Med., MLU, Halle, Germany.

The TNF α is an important cytokine in the complex signalling pathway involved in the development of atherosclerosis. We studied the interaction of the TNF α mRNA expression in monocytes of human peripheral blood (competitive RT-PCR), a promoter polymorphism (PM) of TNF α (G-238A; SSCP) and their association with coronary atherosclerosis. In this study 256 patients with angiographically confirmed diagnosis were involved: 128 patients with severe coronary atherosclerosis (CAD; 81 males, average age: 50.3y) and 128 patients without any coronary symptoms (WAS; 83 males, average age: 50.4y) as controls. As proposed by recent studies the mRNA expression of TNF α was shown to be elevated in the CAD compared to the WAS group (7.4 vs. 6.7ag/cell; n.s.). In order to prove the role of the genetic background we examined the dependence of the mRNA expression on the G-238A PM: Whereas the mRNA expression in WAS patients was only slightly increased in dependence on the genotype (AG+AA: 7.4 vs. GG: 6.6ag/cell) the mutation carriers among the CAD patients showed significantly higher expression than wildtype carriers (AG+AA: 12.6 vs. GG: 6.9ag/cell, $p < 0.012$). An analysis of the genotype distribution of the G-238A PM revealed no significant differences in the frequency of AG+AA-carriers (CAD: 0.09 vs. WAS: 0.12). However the evaluation of the importance of this PM for the early development of severe CAD described by an early age of onset (<45y) revealed significant changes in genotype distribution. The CAD patients with an early age of onset were significantly more often mutation carriers than CAD patients who exhibit coronary symptoms at higher age (<45y vs. >45y: 0.18 vs. 0.02; $p < 0.012$). This result underlines the importance of the mutant genotypes AG+AA of the G-238A PM for having a more pronounced mRNA expression and also for the development of coronary symptoms at younger age (<45y). Investigating the role of the message expression of TNF α in the development of coronary atherosclerosis the importance of the genetic background on message level should always be taking into consideration.

Bioinformatic screening of xenobiotic responsive elements in search of downstream targets of aryl hydrocarbon receptor: Identification of DMRT1 as a candidate. H. Fujita¹, R. Kosaki², T. Suzuki¹, T. Takahashi¹, K. Kosaki¹. 1) Dept of Pediatrics, Keio Univ Sch Med, Tokyo, Japan; 2) Dept of Genetics, Saitama Children's Medical Center, Saitama, Japan.

In utero exposure to dioxins affects male external genital development in rodents. The effects of dioxins are mediated via the aryl hydrocarbon receptor and is negatively regulated by the aryl hydrocarbon receptor repressor. The dioxin-aryl hydrocarbon receptor complex binds to the cognate xenobiotic responsive elements in the promoter regions of the target genes. We recently demonstrated weak but significant association between micropenis and the Pro185Ala polymorphism of the aryl hydrocarbon receptor repressor (Fujita et al, Teratology 2002). In order to delineate downstream target genes of the dioxin receptor, we systematically surveyed the promoter sequences of various genes (*DMRT1*, *SOX3*, *SOX9*, *SF-1*, *GATA4*, *WT1* and *FGF9*) implicated in genital development, searching for the core pentamer consensus sequence GCGTG of the xenobiotic responsive element. Two elements were present upstream of the major transcription start site of *DMRT1*, a recently described gene that is specifically expressed in the gonads and is required for postnatal testis differentiation, whereas no more than one element existed adjacently in the upstream of the putative transcription start sites of the remaining genes. The density of the elements was significantly higher than that expected by chance. Comparative genome analysis of the *DMRT1* sequences revealed that these putative xenobiotic responsive elements were conserved in the rat genome. Furthermore, the two putative xenobiotic responsive elements coincide with the activating segment of the promoter documented by other group using site-directed mutagenesis (Lei et al, Biol Reprod 2002). We suggest that the putative xenobiotic responsive elements of the *DMRT1* gene may be functionally relevant and that *DMRT1* may be the downstream target of the aryl hydrocarbon receptor.

Human LINE-1 retrotransposons are translated by an unconventional mechanism. *R.S. Alisch, J.V. Moran.* Dept Human Gen & Internal Med, Univ Michigan, Ann Arbor, MI.

Long Interspersed Elements (L1s) are abundant retrotransposons and comprise ~17% of human DNA. Retrotransposition-competent L1s are 6.0 kb and contain a 5' untranslated region (UTR), two non-overlapping open reading frames (ORF1 and ORF2), and a 3' UTR that ends in a poly (A) tail. ORF1 encodes a RNA binding protein (ORF1p), whereas ORF2 encodes a protein (ORF2p) with both endonuclease (EN) and reverse transcriptase (RT) activities. Previous studies demonstrated that both proteins are required for efficient retrotransposition in cultured human cells. The L1 transcript is unusual because it contains two distinct ORFs. Thus, an understanding of how ORF2 is translated will provide fundamental insight about unconventional translation mechanisms that exist in human cells. To identify cis-acting sites important for ORF2 translation, we created a deletion series of the 66 nucleotide intervening sequence (IS) that lies between ORF1 and ORF2. Partial or complete deletion of the IS had little or no effect on retrotransposition. Similarly, frameshift mutations positioned upstream of the putative ORF2 initiation codon also had no effect on retrotransposition. By contrast, the introduction of a stop codon (L11X) near the presumptive amino terminus of ORF2p completely abolished retrotransposition. These data suggest that the translation of ORF2p does not rely on the IS, that ORF2p is not made an ORF1/ORF2 fusion protein, and that ORF2p initiates prior to codon 11. To further elucidate how ORF2p translation is initiated, we mutated the putative AUG initiation codon to AUA or CCC. Remarkably, these mutations only slightly reduced retrotransposition (~45% of wild-type). Subsequently, we proved that retrotransposition of the mutant constructs was not promoted by endogenous sources of RT. Finally, all of the above constructs demonstrated similar abilities to retrotranspose in Chinese Hamster Ovary cells. Thus, because there is no evidence for splicing of L1 mRNA, and there are no other candidate initiation codons (AUG, GUG, CUG) near the presumptive amino terminus of L1 ORF2, our data strongly suggest that ORF2 may initiate translation in an AUG independent manner.

The LZ Domain of ORF1p is Required for Human L1 Retrotransposition. *A.E. Hulme, J.N. Athanikar, J.V. Moran.*
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Retrotransposition-competent L1s (RC-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 ORF1 encoded protein (ORF1p) co-localizes with L1 RNA in cytoplasmic ribonucleoprotein particles, which are proposed retrotransposition intermediates, yet its exact function remains unknown. The carboxyl terminus of ORF1p is highly basic and contains a number of amino acids that are conserved among mammals, some of which may function in RNA binding. In contrast, 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, but the LZ is absent from rat and mouse L1s. Therefore, it is uncertain whether the LZ domain is required for retrotransposition.

Here, we show that pairwise missense mutations (Leu to Val) of the four leucine residues constituting the LZ abolish L1 retrotransposition in cultured human cells. We hypothesized that this defect was due to the inability of the LZ mutants to form homomultimers. To investigate this possibility, we expressed either wild type or LZ mutant ORF1p containing a T7 gene 10 epitope tag on their respective carboxyl termini in HeLa cells. Whole cell extracts then were treated with increasing amounts of glutaraldehyde to cross-link tightly associated proteins, and the ability of ORF1p to form homomultimers was examined on SDS-PAGE gels by Western blotting. Unexpectedly, our data demonstrated that the LZ mutants could form homomultimeric complexes under the same conditions as wild type ORF1p. We next conducted GST pulldown assays to investigate ORF1p heteromultimeric complex formation. Our data revealed that wild type and LZ mutant ORF1p could interact either as homodimers or heterodimers. Thus, we conclude that mutations in the LZ domain eliminate retrotransposition, but still allow ORF1p-ORF1p interaction. These paradoxical findings show that multimer formation of ORF-1p is not sufficient for retrotransposition, and suggest that the LZ domain has another role in retrotransposition.

Identification and characterization of an active HERV-E gene (ERVE1) that is expressed in the pancreas and thyroid. *J. Sugimoto*¹, *T. Shiroma*², *G. Okahara*², *T. Oda*¹, *F. Kanaya*², *Y. Jinno*¹. 1) Mol Biol/Okinawa-Asia Res Ctr, Univ Ryukyus Sch Medicine, Nakagami, Okina, Japan; 2) Department of Orthopedic Surgery, University of the Ryukyus, Okinawa, Japan.

To elucidate possible physiological functions of human endogenous retroviruses (HERVs) and their role in the pathogenesis of human diseases, we have developed a strategy to identify transcriptionally active HERV genes. By this approach, we have identified and isolated an active HERV-E gene (ERVE1) that was mapped to 17q11. Northern blot analysis revealed in the pancreas (and thyroid) two major transcripts, 3.3 and 4.1kb in size, associated with 500- to 600-nucleotide-longer minor bands. The 3.3kb cDNA clone, identified by cDNA library screening and RACE, contained an open reading frame of 660 nucleotides, translating into a protein of 219 amino acids. In vitro translational analysis of the cDNA revealed a 25kDa protein, consistent with the predicted molecular weight of the gene. Although the deduced amino acid, from ERVE1 was unique and did not possess a high degree of homology to sequence in the non-redundant protein and nucleotide databases, preferential expression in pancreas and thyroid gland tissues may suggest a role for this gene in physiological functions common to these tissues. In addition, while the activity of these retrotransposons is usually suppressed by DNA methylation and/or other mechanisms, this active ERVE1 may serve as an appropriate material for investigating of gene regulation.

FMR1 mRNA derived from premutation alleles is translated with reduced efficiency. *C. Bagni*¹, *F. Tassone*², *B. Primerano*¹, *A. Ludwig*², *C. Mamay*², *R. Hagerman*³, *P. Hagerman*². 1) Department of Biology, Università di Roma, Tor Vergata, Rome, Italy; 2) Department of Biological Chemistry, University of California at Davis, Davis, California; 3) M.I.N.D. Institute, University of California Davis Medical Center, Sacramento, California.

Reduced levels of the FMR1 protein (FMRP) have been observed in some male carriers of the fragile X premutation despite the presence of elevated FMR1 message levels. In order to determine whether translational efficiency is reduced for FMR1 mRNA derived from premutation alleles, we have determined the polysome profiles for FMR1 mRNA in twelve lymphoblastoid cell lines derived from individuals carrying unmethylated alleles that range from 60 to 500 CGG repeats. FMR1 message and FMRP expression levels were determined in each cell line by quantitative (fluorescence) RT-PCR and Western Blot, respectively. The percent of the mRNA on the polysomal fraction (PMP) was also calculated. Preliminary results demonstrate reduced polysomal loading of the FMR1 mRNA within the premutation range, indicative of decreased translational efficiency. The decrement in polysome loading is more pronounced with increasing CGG repeat number, consistent with both lowered FMRP levels and the tendency to more significant clinical involvement in the upper portion of the premutation range.

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Expression of the Human and Murine 5'-(CGG)_n-3'-binding proteins CGGBP1. *F. Naumann, B. Schmitz, W. Doerfler.* Institute of Genetics, University of Cologne, Cologne, Germany.

The fragile X syndrome, a frequent cause of mental retardation in humans, is commonly associated with an unstable CGG repeat within and hypermethylation of the promoter of the FMR1 gene. Expansions in trinucleotide repeat sequences in the human genome have been linked to a number of diseases in humans. We have isolated a 20 kDa protein (CGGBP1) which binds specifically to CGG repeats, either in synthetic oligodeoxyribonucleotides or in the 5'-upstream region of the human and the murine FMR1 gene. CGGBP1 does not bind to completely methylated repeats. When overexpressed after transfection into HeLa cells, CGGBP1 can inhibit the activity of the FMR1 promoter. Until June 2002, screening of protein databases with the sequence of CGGBP1 has not revealed a related sequence. We have cloned and determined the sequences of the human and murine genes. Sequence comparisons with available EST clones revealed that both translated and a high proportion of the long 5'- and 3'-untranslated regions are highly conserved in mammals. Furthermore, the promoter of the murine CGGBP1 gene is highly conserved over 450 bp upstream of exon 1. Due to alternative polyadenylations, messages of 1.2 and 4.4 kilobases are expressed at different ratios in human and murine embryonic, fetal and adult tissues. We inserted fragments of varying lengths from the 5'-flanking region of the human CGGBP1 gene upstream of the firefly luciferase gene and assayed for luciferase activity after transfection of these constructs into HeLa cells. Luciferase activity decreased strongly when constructs with less than 200 bp upstream of exon 1 were used. Although sequence analyses of the highly conserved 5-UTR had suggested a role in posttranscriptional control, no difference in luciferase activity could be observed in transfection experiments using HeLa cells with constructs carrying the 5-UTR. The detailed structural analyses described here were performed to obtain a solid base for further work on the biological function of the CGGBP1 gene which is still unknown. [This research was supported by the Center for Molecular Medicine Koeln TV13].

Genomic structure and splice variants of PPP2R2B, the gene associated with spinocerebellar ataxia type 12 (SCA12). *S.E. Holmes¹, H. Hwang¹, E. O'Hearn², S.E. Antonarakis⁵, S. Chip⁶, S. Strack⁶, C.A. Ross^{1,3,4}, R.L. Margolis^{1,4}.* 1) Dept of Psychiatry,; 2) Dept of Neurology,; 3) Dept of Neuroscience,; 4) Program in Cellular and Molecular Medicine, Johns Hopkins Univ Sch Med, Baltimore, MD; 5) Div of Medical Genetics, Univ of Geneva,CMU, Switzerland; 6) Department of Pharmacology, University of Iowa.

SCA12 is associated with an expansion mutation of a CAG repeat located within the gene encoding PPP2R2B, a regulatory subunit of the trimeric phosphatase PP2A. The repeat was in the 5' flanking region of the originally published PPP2R2B cDNA. Using a combination of RT-PCR, ESTs in GenBank, and sequence analysis algorithms, we have now identified additional exons and multiple splice variants of PPP2R2B. The gene contains nineteen known exons, with exons 12-19 contained in all splice variants, and exons 1-11 included in various combinations. The repeat is located in exon 10, and depending on the transcript variant, is therefore alternatively in an intron, in 5'UTR, and potentially within an open reading frame. Our cell model results suggest that the region around the repeat can function as a promoter; but the presence of splice variants containing additional upstream exons suggests that other promoters probably also exist. Translation of the variants indicates that there are at least 6 different potential N-termini of the protein, potentially affecting the subcellular localization of the PP2A enzyme (Strack et al., 2002). While we have shown that an increase in repeat length leads to increased expression of PPP2R2B in cell culture, it is possible that the expansion could lead to pathogenesis through alternate mechanisms, including altered splicing, a toxic effect of mRNA, or a protein with an expanded serine repeat. Strack, S. et al., *J. Biol. Chem.* 2002, 277: 20750-20755.

Microarray analysis of gene expression in SCA1 transgenic mice. *H.G. Serra¹, C.E. Byam¹, J.D. Lande¹, S.K. Tousey¹, H.Y. Zoghbi², H.T. Orr¹.* 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.

Spinocerebellar ataxia type 1 (SCA1) is a neurodegenerative disease caused by the expansion of a glutamine repeat within the SCA1-encoded protein, ataxin 1. Although ataxin 1 is ubiquitously expressed, the disease affects a specific subset of neurons including the Purkinje cells of the cerebellar cortex and neurons within the brainstem. We used genome-wide oligonucleotide microarrays to characterize gene expression patterns in the cerebellum of three different SCA1 transgenic mouse lines at 5 weeks of age; that is, before the onset of the neurological disease phenotype. We used an ataxic transgenic line expressing one expanded allele with 82 CAG repeats (B05 line); a non-ataxic transgenic line expressing wild type SCA1 allele with 30 repeats (A02 line) and a non-ataxic line that expresses ataxin 1 with 82 CAG repeats only in Purkinje cell cytoplasm (K772T line). We reasoned that we could perform a genetic analysis by comparing the ataxic line (B05) with the two non-ataxic lines (A02 and K772T) followed by a comparison between the two non-ataxic lines (A02 and K772T). The set of genes obtained in the last comparison (A02 and K772T) is not likely to play an important role in the disease process; therefore, we subtracted these genes from those obtained in the first two comparisons (B05 vs A02, and B05 vs K772T). We were able to identify a set of eleven genes (5 novel and 6 known genes) highly expressed in cerebellar Purkinje cells, the most affected site in SCA1. The set of known genes includes Purkinje cell protein 1 (PCP1), Glutamate transporter (EAAT4), Inositol 1,4,5-triphosphate receptor 1 (IP3R1), Homer-3, G-Substrate and Carbonic anhydrase related sequence (CARP). Of the six known genes, four (Homer-3, G-Substrate, EAAT4, IP3R1) can be placed within the glutamate-signalling pathway. In summary, this work provides a broad-based information on the molecular mechanism involving SCA1 pathogenesis and permits a further insight on the complexity of SCA1 disease.

Systematic identification and characterization of eye-expressed transcripts: novel candidate genes for eye diseases. *S. Banfi*¹, *I. Conte*^{1, 2}, *M. Lestingi*¹, *C. Ziviello*¹, *C. Vitiello*¹, *D. Circolo*¹, *M. Pugliese*¹, *M.G. Miano*², *A. den Hollander*³, *F. Cremers*³, *A. Ballabio*¹, *A. Ciccodicola*². 1) Telethon Institute of Genetics and Medicine (TIGEM), Naples, Italy; 2) Institute of Genetics and Biophysics "Adriano Buzzati-Traverso" (IGB) CNR, Naples, Italy; 3) University Hospital of Nijmegen, The Netherlands.

Inherited eye diseases are a common cause of visual impairment in children and young adults. The molecular causes of the majority of the more than 200 eye disorders recognized thus far have not been elucidated yet. We propose to identify and characterize, in a systematic manner, a number of human genes which are specifically or predominantly expressed in the eye and can therefore represent candidates for inherited eye disorders. We selected these cDNAs both by using bioinformatic tools (in silico differential expression screening using the public EST databases) and by using subtractive human cDNA libraries enriched for retina- and retinal pigment epithelium (RPE)/choroid-specific cDNAs. We are currently carrying out a more detailed characterization of these cDNAs including mapping assignment in the human genome, identification of the full-length human transcripts, and detailed analysis of the expression patterns by RNA in situ hybridization on mouse tissues. So far, we have selected 100 putative eye-specific that have been analyzed by semiquantitative RT-PCR PCR to verify their expression level in retina. Forty of these cDNAs turned out to be specifically or predominantly expressed in retina and/or RPE. Furthermore, for many of them, we have characterized the genomic structure and we have isolated the full-length cDNA. We are analyzing the possible involvement of these candidate genes in eye inherited disorders by carrying out extensive mutation analysis, by DHPLC approach, in a large collection of retinal degeneration patients, including both familial and sporadic cases.

DNA-protein and protein-protein interactions by DNA affinity capture-LC/MS/MS. *T.K. Bane¹, J.M. LeBon¹, T.D. Lee², A.D. Riggs¹.* 1) Division of Biology, City of Hope Nat. Med. Ctr, Duarte, CA; 2) Division of Immunology, City of Hope Nat. Med. Ctr, Duarte, CA.

DNA-protein interactions are at the foundation of gene regulation and epigenetic phenomena such as X Chromosome Inactivation (XCI). Epigenetic regulation is the mechanism for the selective activation or inactivation of genes within cells. Disruptions of epigenetic states represent human disease phenotypes, especially developmental defects and tumorigenesis. Numerous studies have shown that 5-methyl-cytosine strongly affects DNA-protein interactions and inhibits transcription when present in promoters. XCI, which results in the heritable inactivation of one X chromosome early in mammalian embryogenesis, is maintained by the enzymatic hypermethylation of cytosine at CpG sites on the inactive X chromosome. A gene called XIST, which is expressed only from the inactive X chromosome, controls the initiation of XCI. This study investigates the effect of cytosine methylation on protein binding to the human XIST minimal promoter in nuclear extracts. We are using DNA affinity capture (DACA) combined with both Surface-Enhanced Laser Desorption/Ionization-Time of Flight Mass Spectrometry (SELDI-TOF MS) and differential isotopic labeling combined with nano-LC/MS/MS to investigate DNA-protein and protein-protein interactions. We have successfully carried out DACA-TOF mass spectrometry using DNA fragments bound to a surface to analyze 1) sequence specific DNA-protein interactions and 2) the DNA-protein, protein-protein interactions specific to methylated versus unmethylated fragments of the human XIST minimal gene promoter. Comparisons between mass spectra indicate that in general, protein complexes that form on the unmethylated capture molecule are strongly repressed on the methylated molecule. Currently, this method does not provide conclusive identification of the bound proteins. Therefore, we have carried out differential isotopic labeling to examine the differences in our two protein samples enriched by DACA. We have found that the ICAT system is capable of providing significant purification, relative quantitation and identification of the proteins involved.

CTCF binding sites and developmental changes in DNA methylation at the 5' end of a gene that escapes X inactivation. *C.M. Disteche¹, G.N. Filippova², J-P. Truong¹, K. Tsuchiya³*. 1) Department of Pathology, University of Washington, Seattle, WA; 2) Fred Hutchinson Cancer Research Center, Seattle WA; 3) Department of Pathology, Vanderbilt University, Nashville, TN.

SMCX/Smcx is a gene that escapes X inactivation in human and mouse. Our previous studies have shown that Smcx can be completely inactivated in cells of early mouse embryos, whereas the gene escapes inactivation in adult cells, indicating that genes that escape X inactivation undergo a process of reactivation during development. In mouse the CpG island of Smcx is located near a gene subject to X inactivation. Our analysis of the CpG island of Smcx by electrophoretic mobility shift assays showed that two binding sites for the CTCF insulator were located in the region, suggesting that these sites might help establishing chromatin domains. We examined the methylation pattern of Smcx CpG island during development, using the bisulfite method. Although no methylation was found in adult tissues, as expected for a gene that escapes X inactivation, partial CpG methylation was found in Smcx CpG island of early female mouse embryos. However, CpG methylation was absent from the CTCF binding sites, suggesting that CTCF might interfere with the establishment of DNA methylation and thus protect Smcx from stable X inactivation.

DNMT1 and DNMT3b are not required for maintenance of XIST repression in human cancer cells. *L.V. Pereira, L.R. Vasques, M. Soukoyan.* Dept Biol, Inst Biociencias, Universidade de Sao Paulo, Sao Paulo, SP, Brazil.

The process of X chromosome inactivation (XCI) in human and mice involves expression of the XIST/Xist gene from the inactive X chromosome (Xi) and repression of the XIST/Xist allele on the active X chromosome (Xa). Repression of the XIST/Xist gene on the Xa upon differentiation has been associated with methylation of its 5' region. In mice, Dnmt1 has been shown to be involved in the methylation and transcriptional repression of Xist on the Xa. In human cancer cells, DNMT1 and DNMT3b cooperate to maintain global DNA methylation and gene silencing. We examined the role of human DNMT1 and DNMT3b in maintaining XIST gene repression on the Xa in the human cancer cell line HCT116. Methylation of the XIST promoter and XIST transcriptional repression is sustained in DNMT1-, DNMT3b- and DNMT1/DNMT3b-deficient cells. In contrast, global DNA demethylation of these cells with 5-aza-2-deoxycytidine leads to XIST expression. We conclude that additional DNA methyltransferases are involved specifically in the process of maintenance of XIST repression in these human cells.

Program Nr: 979 from 2002 ASHG Annual Meeting

Comparison of methods for recruitment from a high-risk cancer clinic. *T.M. Friebe, R.A. Beutler, S. Lee, B. Bernhardt, K. Helzlsouer, C.A. Griffin.* Mid-Atlantic Cancer Genetics Network, Johns Hopkins University, Baltimore, MD.

The Mid-Atlantic Cancer Genetics Network (MACGN) is one site of the nationwide Cancer Genetics Network (CGN). The CGN was established as an infrastructure to facilitate research in clinical cancer genetics. MACGN targets individuals from cancer risk assessment clinics for recruitment into the CGN registry. Two methods of recruitment into the registry were compared over an eight month period. A passive recruitment technique was used during the first four months of recruitment, which involved a brief mention of the MACGN program and distribution of a brochure. An active recruitment method was used during the second four month period, in which MACGN staff approached patients and initiated a brief discussion of the MACGN program, after which patients were given the opportunity to decline participation, to enroll then and there, or to take an enrollment packet home. One hundred fifty-eight patients were seen in the clinic during the first four month period and 142 in the second four month period. The demographics and clinical characteristics of the patients who were approached using the two methods were similar in age, ethnicity, level of education and marital status. The change to the active recruitment method resulted in a four fold increase in enrollment by invitees (from 15.6% to 67.4%). This increase shows the importance of personal contact with potential participants as well as the value of allowing time for research staff to describe the program in detail and answer questions that potential research participants may have. Funded by NCI/NIH grant CA 78148-04.

Attitudes of adolescents on genetic screening and testing. *D.N. Abuelo¹, A. Harel², A. Kazura³*. 1) Pediatrics, Rhode Island Hosp, Providence, RI; 2) Brandeis University; 3) Psychiatry, Hasbro Children's Hospital, Providence, RI.

Purpose: To examine adolescents' attitudes toward screening/testing for hereditary disorders. **Methods:** A survey was distributed to 672 students grades 10 to 12 attending a suburban high school. It included information about three diseases: Tay-Sachs, hypercholesterolemia and familial breast cancer. A questionnaire explored their attitudes toward testing for these diseases. **Results:** 361 questionnaires were returned, a 54% response rate. Mean age of participants was 17 ± 1 years. Most girls (67%) wanted to be tested for familial breast cancer. Girls were significantly more interested than boys in testing for hypercholesterolemia. Individuals in the ethnic risk groups for Tay-Sachs disease were significantly more willing to be tested than those not in the ethnic risk groups, but only 33% would either "definitely" or "probably" wish to be tested. Girls who had a relative with breast cancer were significantly more willing to be tested than other girls. Similarly, individuals with a family history of hypercholesterolemia were significantly more interested in testing than those without a family history. About 81% of students with a positive family history had never been offered cholesterol testing. Only about 25% of participants stated that their attitude about genetic testing might result in discrimination by insurance companies or employers. **Conclusions:** Adolescents are receptive to educational efforts about genetic diseases and many are interested in having testing or screening for genetic diseases. The main motivator is having someone in the family affected by the disease in question.

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Long-term re-contact for genetics services. *R.R. Lebel, B. Stradling, B. O'Donnell, D. Skipper, C. Yarshen, C. Malik.*
Genetics Risk Assessment Service, Hinsdale Hospital, Hinsdale, IL.

Modern computerized databases allow for storage of numerous details, including family history information that may be encoded in such a way as to be retrieved to assess risk for complex disease. We may know, for example, that the husband of a woman seen for advanced maternal age has three paternal aunts with breast cancer, placing him and other family members at risk. Searching such a database for families with multiple members affected by cancer of the breast, ovary, colon, prostate or uterus, we identified hundreds of people who might benefit from re-contact for consultation to discuss new technology for the assessment of genetic risk factors. We found not only that many people who have not been seen for ten years are difficult to locate, but also that many of those contacted have limited interest in pursuing consultation and testing. We will present a detailed discussion of the results of these efforts, which show that approximately 5% of the charts reviewed were found to have potential for useful cancer genetics consultation. We will explore some of the psychosocial and ethical aspects of this innovative approach to the duty to re-contact.

Breast cancer families; risk estimation for clinical practice. *C.J. Van Asperen¹, M.A. Jonker², J.E. Van Diemen-Homan¹, E. Bakker¹, M.H. Breuning¹, J.C. Van Houwelingen², G.H. De Bock³.* 1) Departments of Human and Clinical Genetics; 2) Medical Statistics; 3) Medical Decision Making, Leiden University Medical Center, The Netherlands.

Background At present, the majority of women from breast cancer families tested for BRCA1 and BRCA2 mutations will receive a negative test result, although they were referred by a family cancer clinic. For this group of women from high-risk families, risk can only be estimated by examining their pedigree on account of which referral to standard protocols for surveillance takes place. Because of the various kind of counselees from breast cancer families (healthy and affected) it is important to choose for a consistent risk estimation in order to be able to compare the amount of risk in families visiting family cancer clinics. We propose a new method for risk estimation in breast cancer families by analyzing pedigrees from one point of view.

Methods Family histories of 200 consecutive counselees were analyzed. Risks were noted for a (fictive) healthy sister of the breast or ovarian cancer patient, most nearest to the counselee. Risks were subsequently correlated with common risk estimates, like the Claus tables.

Results Out of the 200 family histories, 99 (49.5%) did not fit in the Claus tables only. In comparison with original Claus tables this new method demonstrated higher sensitivity (0.83 vs 0.52) and lower specificity (0.24 vs 0.48) for mutation detection. Both models had a low positive predictive value of 0.2.

Conclusion This method provides a new standard in family cancer clinics as more clinical information is used. Only a model with higher specificity for mutation detection in breast cancer families can reduce the high number of uninformative DNA test results.

The utility of subtelomeric and chromosome testing in patients with congenital heart defects. *K.L. Russell, G. Wernovsky, E. Goldmuntz, J.W. Gaynor, I.D. Krantz, J.E. Ming, S. Saitta, D.M. McDonald-McGinn, L. Celle, N.B. Spinner, E.H. Zackai.* The Children's Hospital of Philadelphia (CHOP) and University of Pennsylvania School of Medicine, Philadelphia, PA.

Here we emphasize the importance of subtelomeric testing in addition to chromosomal analysis in infants with CHD as a means to discern underlying diagnoses and utility in prognostication. Over a one year period (2001), we were asked to evaluate 32 infants with congenital heart disease in the Cardiac Intensive Care Unit at CHOP, in whom there was a question of dysmorphism and/or other organ system involvement. In 12/32 patients, the heart defect was determined to part of a known syndrome: 22q11.2 deletion (4); Noonan (3); CHARGE (1); VATER (1); heterotaxy (2); hemifacial microsomia (1). In 6/32 patients the dysmorphism was minor, if present at all. They were felt to have isolated non-syndromic heart disease after normal chromosome and 22q11.2 studies (and normal Williams probe done in 1). Fourteen/thirty-two children were thought to be dysmorphic but their features did not fit a known syndrome. Chromosomal analysis identified an unbalanced translocation in 2 children. A patient with a double outlet right ventricle and interrupted aortic arch had a 4 way maternally derived unbalanced translocation involving chromosomes 5, 6, 10, 18 (p13;q12;q11;q12). A patient with truncus arteriosus had a de novo unbalanced translocation 45,XY,der(5),t(5;22)(p15;q22.2).

Subtelomeric testing was performed on 11/12 of the remaining undiagnosed patients. Four patients were found to have a subtelomeric rearrangement. These included a de novo 9q deletion; a de novo 1p deletion; 11q deletion; a maternally derived 4q deletion. Thus, examination and laboratory testing was successful in detecting the etiology of congenital heart disease in 24/32 (75%) patients. Having a diagnosis is important for recurrence risk counseling as well as for prognostication and one must now factor in the impact of previously cryptic subtelomeric rearrangements on the long term neurologic outcome following cardiac repair.

Phenotype/karyotype correlation in inv dup(15) marker chromosomes: guidelines for evaluation. *S.E. Thompson, M.D. Graf, S. Schwartz.* Center for Human Genetics and Department of Genetics, Case Western Reserve University and University Hospitals of Cleveland, Cleveland, OH.

The inverted duplicated chromosome 15, [inv dup(15)] is the most common extra structurally abnormal chromosome, comprising approximately 57% of all detected marker chromosomes. The inv dup(15) varies both in size and associated phenotype, ranging from normal to mental retardation, to what has been deemed the inv dup(15) syndrome. With the advent of FISH in the early 1990s, the inv dup(15) has been better identified and defined cytogenetically and molecularly. However, even with the advances in technology, there has been continued debate concerning phenotype/karyotype correlation in the inv dup(15). There has not been complete correlation between cytogenetic or molecular characteristics and the resulting phenotypes, though presence or absence of the PWS/AS critical region (PW/ASCR) has provided the most consistent findings. In this study we report on clinical, cytogenetic, and molecular aspects of a total of 206 cases of inv dup(15) to further characterize the phenotype/karyotype correlation of the inv dup(15) syndrome. Factors studied included phenotype, marker size based on FISH analysis, ascertainment, familial inheritance, and presence of mosaicism. The data showed a significant association between the presence of the PW/ASCR and an abnormal phenotype. Based on this data, recommendations regarding genetic counseling of prenatally detected inv dup(15) marker chromosomes have been developed. From these studies we have gained additional insights concerning the implication of the inv dup(15) chromosome and can conclude that: (1) this is the largest and most definitive study correlating the size of the marker chromosome, based on FISH, and phenotypic consequences; (2) the vast majority of patients with large markers could be classified as having the inv dup (15) syndrome demonstrating MR and seizures and/or autism.; (3) the size of the marker could be correlated to phenotype, inheritance and ascertainment, but not to the presence of mosaicism; and (4) we have developed specific guidelines for approaching these marker chromosomes if ascertained prenatally.

TEACH: Development and evaluation of a computer program for collecting family cancer history. *C.A. Griffin, Q. Qu, R.A. Beutler, J. Sollenberger.* Mid-Atlantic Cancer Genetics Network, Johns Hopkins, Baltimore, MD.

Recognition of an individual's increased risk for development of cancer due to his/her personal or family history of cancer requires collection of family history in sufficient detail. A major obstacle to obtaining the family history is time required to ask the individual about relevant members of the family. Additional barriers include time to record the information and to display it in a quickly usable form. We hypothesize that development of a tool that can be used easily by an individual and which immediately displays the data in a pedigree will facilitate collection, recording, and health care provider evaluation of the family history. We have developed a computer program (TEACH) into which an individual enters personal and family history of cancer in four generations. TEACH consists of original software written in Visual Basic with an Access database back-end loaded on a freestanding secure touchscreen computer placed in a doctors office, waiting room, or clinic. The program automatically draws a pedigree from the information and prints a hard copy for immediate use. The first version of the program was tested on 20 volunteers (mostly Hopkins employees and students) and 20 volunteer patients at the Sidney Kimmel Cancer Center. The testing group was highly educated (45% held graduate degrees). Average time required to enter information was 17.9 minutes, and all testers stated that this was an acceptable length of time. The testing group had little concern about using patient ID numbers as identifiers in the program (4.6 on a 5-point Likert scale) but were less comfortable using social security numbers (3.4). Testers were slightly more comfortable entering family members names into the program (3.5), but overall were only moderately concerned (2.6) about the security of information entered into the program. Testers felt generally comfortable (4.1) with having the completed pedigree placed in their charts. We conclude that TEACH can function as a tool to collect family history, and are in the process of evaluating its use in academic, community, and private practices. Supported by NCI/NIH grant CA 78148-04.

Genetic counseling referral and perceived benefit for New Jersey Fetal Abnormalities Registry (NJFAR)

subjects. *K. DePrince¹, A.J. Fisher¹, J.C. Canterino¹, R.L. Fischer¹, R.J. Librizzi², J.C. Smulian¹ and New Jersey Fetal Abnormalities Registry.* 1) University of Medicine and Dentistry of NJ, New Jersey, USA; 2) Jefferson Medical College, Pennsylvania, USA.

NJFAR collects information on pregnancies of NJ residents after 14 6/7 weeks gestation in which a fetal structural abnormality has been identified or suspected by Maternal-Fetal Medicine specialists. In a sub-set of these cases, women provide consent and actively participate in an extensive interview about medical history, family history, pregnancy course and outcome. Subjects are asked about genetic counseling referral and its outcome during the interview. This study was designed to determine 1) the frequency of genetic counseling referral and delivery; 2) the venue for genetic counseling when obtained; 3) potential reasons for not having genetic counseling; and 4) perceived helpfulness of genetic counseling. Responses from 50 consecutively interviewed subjects showed that genetic counseling was not offered in 9 (18%) cases (all with a defect in 1 organ system), was offered but not obtained in 10 (20%) cases (5 with a defect in 1 organ system / 5 with defects in multiple organ systems), and was offered and obtained in-person (n=27) or via telephone only (n=4) in 31 (62%) cases (14 with a defect in 1 organ system / 17 with defects in multiple organ systems). Genetic counseling was perceived as helpful in 27 (87%) of 31 cases when provided. The most common stated reason for not having genetic counseling when offered was having had genetic counseling in a previous pregnancy (3/10; 30%). This study demonstrates that the majority (82%) of NJFAR consented subjects are offered genetic counseling but only 62% actually receive genetic counseling services. Those who have genetic counseling overwhelmingly find the service helpful in dealing with the diagnosis of a fetal structural abnormality. (Supported by the NJ Department of Health & Senior Services and CDC Centers for Birth Defects Research and Prevention).

Prenatal diagnosis for cystic fibrosis: complex counseling and crisis intervention. *R. Babul-Hirji, L. Steele, D. Hinkson, P.N. Ray, D. Chitayat.* The Hosp Sick Children, Toronto, ON, Canada.

Prenatal diagnosis for cystic fibrosis (CF) is usually available either by mutation or linkage analysis. At our centre, patients are tested for 31 of the most common CFTR mutations with a sensitivity of ~87% in Caucasians. Linkage using 1 intragenic STR and 6 extragenic RFLP markers is offered when 2 mutations aren't identified. We present a case of complex counseling where linkage analysis was uninformative. A 36-year-old pregnant female who has a child with CF was seen for prenatal diagnosis (PND) of CF at 16 weeks. Her affected child had antenatal diagnosis of echogenic gut, didn't have postnatal sweat chloride testing and was diagnosed with CF at 16 months. The child had the maternal DF508 mutation while the father's mutation was unidentified. The patient was counseled that PND for CF would be by exclusion testing for DF508. If identified, linkage analysis would be pursued. The patient had amniocentesis and the fetus had the DF508 mutation. Linkage showed the father to be uninformative at all 7 markers. The fetus therefore had a 50% chance of having CF. As well, ultrasound at 20 weeks showed echogenic gut. Factors causing anxiety for this patient included uncertainty of the CF results, u/s finding of echogenic gut, dilemma of terminating a normal pregnancy and deciding to have further testing to refine the risk for CF with possibly terminating outside of Canada due to her gestational age. Counseling aimed at providing her with psychosocial and emotional support, providing accurate information and helping her identify areas for timely decision-making. Such a session presented many challenges: trying not to overwhelm the patient with too much technical information, helping her in her decision-making process by using counselling strategies and crisis intervention and recognizing the need for involvement of other clinical and research labs. Ultimately, further DNA analysis showed the fetus to be a carrier of CF. Follow-up sweat chloride testing at 6 weeks was normal. In light of this case, it is recommended that sweat chloride testing be done at 6 weeks of age on babies with an antenatal diagnosis of echogenic gut or predicted to be unaffected with CF by linkage analysis.

A randomized trial of telephone and in-person breast cancer genetic counseling. *J.O. Culver, A. Helmes, D.J. Bowen.* Fred Hutchinson Cancer Research Center, Seattle, WA.

Telephone genetic counseling may be considered when a patient or relative cannot be counseled in-person. We have conducted a randomized trial to determine whether breast cancer risk counseling in-person and by telephone are equally effective in communicating risk information, increasing breast cancer screening, and decreasing cancer worry. Study participants were recruited through a Seattle network of primary care providers and consisted of 340 women between ages 18 and 64 with no personal history of breast cancer. After completing a baseline survey, participants were randomized to one of three study arms: in-person genetic counseling, telephone genetic counseling, or a control group. Both counseling protocols included a discussion of the participant's Gail model breast cancer risk estimate, genetics concepts, and breast screening recommendations. All participants were asked to complete a 3-month follow-up survey. Forty-five percent of study participants had a relative with breast cancer, including 15% with at least one affected first-degree relative. The average Gail model risk for breast cancer was 9.5% by age 79. Yet, the average reported perceived risk was 30%, roughly 3-fold higher than predicted by the Gail model. At follow-up, the average perceived risk was significantly lower in the counseling arms ($p < .001$), averaging 20% in the in-person arm, 21% in the telephone arm, and 31% in the control group. At follow-up, participants in both counseling arms had decreased levels of cancer worry ($p < .01$) and increased intentions to do breast self-exam ($p = .055$). Most (88%) in the in-person arm and 81% in the telephone arm reported that they liked the counseling. Yet 15% of the participants in the in-person arm and 37% in the telephone arm would have chosen the other method of counseling. Our results show no outcome differences between these two counseling methods except for a slight preference for in-person counseling. Because genetic counseling may often be needed when in-person counseling is impossible or difficult, these results could have implications for clinical and research practice.

Breast cancer: Patients' perception of genetic risk, beliefs, attitudes, and experience within cultural diversity.

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Background: Breast cancer is the most common cancer in women accounting for 19% of all cancers. While the incidence of breast cancer in the Indian subcontinent is lower than in Europe, migrant studies have shown that the incidence of cancer in migrant populations approaches that of the indigenous population within one or two generations and that it is an increasing issue for the South Asian population living in the UK. There is evidence that this increase particularly affects young women (25-50 years). The number of cases of breast cancer in the South Asian population resident in Britain is likely to rise and appropriate services will need to be available for these women. The increasing incidence of breast cancer in the South Asian community makes it extremely important that professionals understand cultural beliefs regarding breast cancer treatment and screening. This will allow improved culturally appropriate communication and better health provision to this group of women.

Aim: The aim of the study is to examine health care pathways, attitudes, beliefs and genetic perception of risk factors in South Asian women with breast cancer compared with non-Asian women. Generic issues with relevance to international health care will be drawn out.

Method: The study comprised of two groups of women attending breast clinic because of a diagnosis of breast cancer. The study comprised 25 South Asian women diagnosed with breast cancer and one individual (doctor, nurse, carer) who was a key influence during the breast cancer experienced. The control comprised 15 white women matched for age and socio-economic class diagnosed within the same time frame and individuals who were their key influence.

The method used as qualitative content analysis of interviews conduction in the appropriate language.

Comparison of Web based vs. hospital based retrospective recruitment of participants for a craniosynostosis study. *N. Isaac¹, T.H. Beaty², B. Carson³, E.W. Jabs¹, J. Richtsmeier⁴, C. Vander Kolk³, A.F. Scott¹, S.A. Boyadjiev Boyd¹.* 1) Dept. Pediatrics/Gen Med, Johns Hopkins Univ, Baltimore, MD; 2) Dept. of Epidemiology, Johns Hopkins School of Public Health; 3) Dept. of Surgery, Johns Hopkins School of Medicine; 4) Dept. of Anthropology, The Pennsylvania State University.

A novel Web based approach was used to recruit families, for a study of genetic and non-genetic causes of craniosynostosis. Invitation letters were sent to 4 of the 7 Web based craniosynostosis peer support groups contacted. These 4 groups (Craniosynostosis and Positional Plagiocephaly, Inc.; CRANIOSUPPORT; FACES; and Lets Face It USA) agreed to post study information on their Web sites. Over the past 18 months we received a total of 152 responses. Families were considered for enrollment based on CAT scan reports confirming craniosynostosis and/or a history of surgical correction of abnormal head shape. A sample collection kit was mailed to the family after review of an e-mail based initial screening questionnaire. Genetic evaluation and CAT scan reports were requested to confirm the diagnosis. A phone interview collecting medical and family history completed the recruitment process. Thirty-one families (20%) have been recruited and provided medical information, photographs, and blood samples. Of these 31, 4 probands were syndromic, 21 had one sutural synostosis and 6 had multiple sutural synostoses. An additional 104 families (68%) indicated an interest in participating and are in different stages of the recruitment process. Only 17 families (11%) ultimately chose not to participate. In comparison, invitation letters were sent to 233 families identified retrospectively through pediatric neurosurgery and plastic surgery clinics. Only 16 of these families have been successfully recruited and an additional 11 families have agreed to participate (12%). Of the 16, 2 probands were syndromic, 10 had one sutural synostosis and 4 had multiple sutural synostosis. Web based recruitment was more efficient than the retrospective hospital based approach. There were no significant differences in sutural involvement between participants of both groups.

Autosomal form of Alport syndrome, a model between dominant and recessive inheritance: implications for clinical practice and genetic counseling. *I. Longo¹, F. Mari¹, C. Pescucci¹, F. Ariani¹, M. Bruttini¹, I. Meloni¹, M. De Marchi², A. Renieri¹.* 1) Dept Mol Biol, Medical Genet, University of Siena, Siena, Italy; 2) Dept Clin Biol Sciences, University of Torino, Torino, Italy.

A comprehensive mutation screening of *COL4A3* and *COL4A4* genes let us detect 8 novel mutations in 6 out of 36 Italian families with a suspicious of autosomal Alport syndrome (ATS) (Kidney Int, 2002). Four mutations of *COL4A3* were found in compound heterozygotes in typical autosomal recessive form, i.e. rapidly progressive nephritis. Three mutations of *COL4A3* and 1 of *COL4A4* were found in simple heterozygotes. Further studies let us identify an additional family in which an heterozygous glycine substitution (p.G1198S) was present both in a 34 year-old female with thin basement membrane at renal biopsy, microhematuria and normal renal function and in the father with impaired renal function. Overall, clinical presentation of simple heterozygotes, including parents of recessive patients, ranges from healthy carriers, through Benign Familial Hematuria (BFH), to autosomal dominant ATS, i.e. slowly progressive impaired renal function. Very interestingly, we investigated a large family (36 members) in which two males progressed to End Stage Renal Failure by the age of 20-23 years and had glomerular basement membrane lesions typical of ATS. The father and the paternal grandfather had an apparently non progressive chronic renal failure. One paternal uncle, 1 paternal aunt and 1 paternal cousin had microhematuria only. Linkage analysis was compatible with the presence of two mutations (genetic compound) in the two probands and one heterozygous mutation both in patients with non progressive renal damage and in patients with microhematuria. Our results show that there are no clear-cut limits between the diagnoses of autosomal dominant and recessive ATS and BFH, but that these phenotypes rather belong to a continuous spectrum of graded severity. The wide range of clinical presentation of carriers of a mutation in either *COL4A3* or *COL4A4* gene makes diagnosis and most important prognosis unpredictable in these patients. Moreover this ambiguity makes it very difficult to predict inheritance in single small families.

BRCA1/2 genetic testing: womens information seeking behavior complementary to the informed consent

procedure. A. C.M. Julian-Reynier¹, C. Cypowyj^{1, 2}, F. Eisinger^{1, 3}, F. Chabal¹, M. Morin^{1,2}, H. Sobol³. 1) Inst Paoli-Calmettes, BP156, INSERM U379, Marseille CDX 9, France; 2) Universit de Provence, Aix en Prce, FranceÆ; 3) Inst Paoli-Calmettes, INSERM E9939, Marseille France.

No data are available on information seeking behaviour (ISB) of women outside the cancer genetic consultation when BRCA testing is considered. The objective of this study was to describe ISB for genetic testing complementary to the genetic consultation and their related factors. A prospective ongoing cohort study (2000-2002) is including all women who attended at one cancer genetic clinics in France after a BRCA1/2 analysis had been proposed. Closed questionnaires were administered before and after the occurrence of the 2nd cancer genetic consultation. This consultation aims to confirm the decision to be tested. Preliminary results were analysed (N=104 ; mean age 47, SD=11; 70% affected by cancer). Prior to the 2nd consultation, 36% of the women had actively looked for information on BRCA1/2 testing compared to 23% afterwards. After multivariate adjustment by logistic regression, the before consultation information seeking behavior was related to a higher satisfaction with the cancer genetic consultation occurring afterwards ORadj (95% CI): 1.04 (.99-1.08) (scale from 0 to 100), with the certainty to be a gene carrier ORadj: 3 (1-8), and with the fact to have a treatment for anxiety of depression ORadj: 4 (1-16). Those who actively looked for complementary information were also more often attending with a family member ORadj: 5 (2-14). The other tested variables (depression, coping, sociodemographic and medical characteristics) were not significant (p>0.05). Sources of information will be detailed These results highlight the fact that women who actively seek information on breast/ovarian cancer genetic testing differ from those who do not, not only for their a priori beliefs but also for their satisfaction with counseling and for their social support.

Attitudes of Haemophilia Affected Families Towards Genetic Counselling. *G.S. Pandey, I. Panigrahi, S.R. Phadke, B. Mittal.* Medical Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, UP, India.

The aims of this study was to evaluate the knowledge of haemophilia in families enrolled in the Lucknow Haemophilia Society and assess their attitudes towards prenatal diagnosis and the possible factors influencing these. Questionnaire comprising of multiple choice and open-ended questions was distributed at the society meetings for assessing knowledge and attitudes. The source of knowledge in most patients was the Hemophilia Society. There was a positive response from majority of the families. Almost all of them had in depth knowledge about the disease and the frequency of its recurrence in their families. There were 28 respondents to the questionnaire assessing knowledge of hemophilia and 17 respondents to questionnaire on prenatal diagnosis (PND) attitudes. The average score was 7.3 out of 15 points. There was a positive correlation between the extent of knowledge and the frequency of attending the monthly hemophilia society meetings. This was evident from the prenatal diagnosis questionnaire, where most of the families showed positive attitude for termination of pregnancies if the fetus is affected. In four families of haemophilia A prenatal diagnosis was carried out on chorionic villous sample using four polymorphic markers in the factor VIII gene. Three families reported for prenatal diagnosis using DNA-based linkage analysis, 2 fetuses were found to be affected while the third one was unaffected. Out of the two affected, one opted for termination while the other family decided to continue with the pregnancy even after intensive counseling. Hemophilia society has an important role in educating hemophilia families and improving uptake of services. Geographic, ethnic, and financial factors may affect PND attitudes and to be kept in mind during genetic counseling. The present study highlights that there is a good understanding of the disease among affected families yet prenatal diagnosis is not readily accepted in our society because of male pro society. Therefore, there should be extensive awareness program, which will bring in more families for prenatal diagnosis.

Parents' use of the Internet as a source of genetic information. *M.I. Roche*¹, *D. Skinner*², *K. Kuczynski*², *R. Schaffer*³. 1) Dept Pediatrics, CB #7220, Univ North Carolina, Chapel Hill, NC; 2) Frank Porter Graham Child Development Institute, Univ North Carolina, Chapel Hill, NC; 3) Dept Anthropology, Univ North Carolina, Chapel Hill, NC.

Clinical experience suggests that parental Internet use to obtain genetic information is widespread but influencing factors have been largely unexplored. Two previous studies, Taylor(2001) and Christian (2001) surveyed families prior to their genetics clinic visit. Over 75% had Internet access but fewer than half had searched prior to their visit. Locating information about their relative's condition in "layperson's terms" ranked as the main goal. Searching occurred more frequently if a child was the proband and if a diagnosis had been made. As part of a longitudinal study to determine how families seek, interpret, and use genetic information, parents were queried about Internet use. The first semi-structured interview, conducted after the initial clinic visit, sought to determine the searching methods used, types of information sought, and families' appraisals of their searches prior to and after their visit to the Pediatric Genetics and Metabolism Clinic at UNC-CH. Sixty-nine interviews were audio-taped, transcribed, and entered into the program, NUD*IST 4.0 for data coding and analysis. The keywords "Internet", "web", and "computer" were searched. The families were representative of the ethnic distribution of the clinic population. Four-fifths of the families searched the Internet both before and after the visit. Preparatory searches using broad terms occurred prior to the visit and enabled families to build a genetic vocabulary, anticipate what to expect during the session, and construct questions. Following a diagnosis, searches focused on potential treatments, services, and support. Parental assessments of their Internet searching were laudatory but guarded and the information's uncertain validity was recognized. Parents' use of the Internet as a source of genetic information was widespread in our population, occurred more often in Caucasian families and augmented traditional methods such as personal contact with health and other service providers. Supported by NHGRI grant #HG02164-03.

Process issues in genetic counseling for hereditary breast and ovarian cancer: Client ratings and session characteristics. *C. Wang, K. Milliron, R. Gonzalez, S.D. Merajver.* University of Michigan, Ann Arbor, MI.

Purpose: Process research in genetic counseling offers a way to assess the effectiveness of genetic counseling and can help identify possible areas for intervention. Our study set out to identify important components of the counseling process and the extent to which they are associated with desired outcomes of genetic counseling. **Methods:** A total of 70 women attending the Breast and Ovarian Cancer Risk Evaluation Program at the University of Michigan completed a mailed questionnaire one week following genetic counseling. Client ratings were obtained on the following: ability to ask questions and extent to which information was explained clearly. Session characteristics examined included complexity of family history (rated by the genetics team) and time spent face-to-face with the client. Outcomes of interest were subjective knowledge, uncertainty reduction, satisfaction with understanding of breast cancer risk, and general psychological distress. **Results:** Client ratings of being able to ask questions was significantly associated with greater subjective knowledge, higher satisfaction, and lower psychological distress. Ratings of the extent to which information was clearly explained was significantly associated with greater subjective knowledge, higher satisfaction, and greater uncertainty reduction. Complexity of family history was negatively associated with uncertainty reduction, such that clients who had family histories that were more complex (i.e., difficult to determine likelihood of a hereditary cancer syndrome) were less likely to feel that counseling reduced uncertainty about their breast cancer risk. Time spent face-to-face with the genetics staff was not found to be associated with any of the genetic counseling outcomes, although it was found to be negatively associated with ratings of how clearly genetic risk information was explained. **Summary:** A greater understanding of processes variables and how they are related to genetic counseling outcomes will enable researchers to identify aspects of the encounter that may be targeted for intervention.

Genetic counseling in a pediatric cancer clinic: results of a pilot program. *K.A. Schneider¹, T. Brown², C. Medeiros Nancarrow¹, C. Recklitis¹, L. Diller¹.* 1) Dana Farber Cancer Inst, Boston, MA; 2) Boston Univ, Boston, MA.

In our pediatric cancer clinic, mothers were invited to participate in a genetic counseling pilot study. All mothers were seen for one visit by the same genetic counselor who collected detailed family histories and categorized families as having low, high or uncertain risk for an inherited predisposition to cancer. Participating mothers completed pre- and post-visit questionnaires about genetics knowledge, cancer worry, emotional well-being, and patient satisfaction. 31 mothers enrolled, submitting 31 pre- and 15 post-visit surveys. Participating mothers had mean age of 40, 90% were married, 84% had more than 1 child, and 65% were college graduates. Family histories had not been discussed with pediatric oncologists in 53% of cases. Childrens diagnoses were: brain tumor (9), lymphoma/non-ALL leukemia (8), Wilms tumor (5), neuroblastoma (5), sarcoma (2), and solid tumors (2). 29/31 (93.5%) cases were low risk, 1 case was high risk (possible Li-Fraumeni syndrome) and 1 case was uncertain risk pending documentation. On a 32-item test, mothers' mean knowledge scores increased significantly after the genetic counseling visit (20.3 to 23.9; $p < .05$). Distress levels measured on the Brief Symptom Inventory (BSI) were above population norms but revealed no negative sequelae from the genetic counseling encounter ($n=13$; pre/post mean global severity index score 68.1 /67.9; $p < .05$). In fact, cancer worry significantly decreased following the genetic counseling visit (8/15 had reduced worry; $p < .05$). Patient satisfaction scores were very high and overall, 14/15 (93%) mothers found the genetic counseling visit to be beneficial. In conclusion, mothers are concerned about the potential genetic implications of their children's cancer diagnoses. One genetic counseling session can identify the rare high risk family, facilitate referrals to specialists, and in most cases, alleviate fears about inherited susceptibility.

Cancer genetic counseling and testing by telemedicine - results of a feasibility study. *J. Permuth-Wey^{1,2}, J.A. Betts², A.B. Cantor^{1,2}, J.P. Krischer^{1,2}, R. Sutphen^{1,2}.* 1) Department of Interdisciplinary Oncology, University of South Florida College of Medicine, Tampa, FL; 2) H.Lee Moffitt Cancer Center and Research Institute, Tampa, FL.

The use of telemedicine for delivery of cancer genetic counseling has the potential to improve accessibility, reduce costs and allow delivery of counseling simultaneously to multiple family members who are geographically distant. We conducted a randomized, crossover study to assess the feasibility and acceptability of delivering cancer genetic counseling services via telemedicine. All individuals presenting for cancer genetic counseling at an NCI-designated Comprehensive Cancer Center were eligible for participation. A brief pre-session questionnaire was used to evaluate previous computer, teleconferencing and research participation experience, in order to examine differences between individuals who agreed to participate in telemedicine versus those who did not. Study participants were randomized into two groups to receive their genetic counseling: 1) face-to-face, or 2) via telemedicine. Participants and counselors were asked to evaluate the experience for each session through a post-session questionnaire which was modified from a previously published instrument. Of 74 eligible subjects, 60 agreed to participate. There were no differences between the 14 who declined participation and the 60 who agreed. Of the 60 participants, 30 received their initial genetic counseling session via telemedicine, and 30 received the session face-to-face. There were no perceived differences between the face-to-face and telemedicine pre-test sessions. Of the 60 participants, 23 proceeded with genetic testing 12 of whom had received the initial session face-to-face and 11 via telemedicine. Based on the crossover design of the study, these 23 individuals received their post-testing genetic counseling session via the opposite method from their initial session. A two-sided test of significance showed no difference in overall satisfaction between telemedicine and face-to-face sessions. This study suggests that telemedicine is perceived as a satisfactory method for the delivery of cancer genetic counseling.

Oral History of Human Genetics Project. *A. Maestrejuan*¹, *M. Meldrum*¹, *E.R.B. McCabe*^{2,3}. 1) Department of History, UCLA; 2) Department of Pediatrics and Human Genetics, David Geffen School of Medicine at UCLA; 3) UCLA Center for Society, the Individual, and Genetics, Los Angeles, CA, 90095.

The second half of the 20th century saw a dramatic growth of human genetics from its very beginnings to the near-completion of the Human Genome Project. The working parameters, scientific assumptions, methodologies and interpersonal networks that were formed just prior to, and during the development of, the field have shaped today's concepts and have prompted the scientific and social questions that drive current research. The Oral History of Human Genetics (OHHG) Project is premised on the assertions that a knowledge of history: will assist today's and tomorrow's practitioners to re-examine the conceptual frameworks and implications of their assumptions and methods; will suggest new directories for research; will inform debate on ethical, legal and social issues critical to genetics; and will inspire students entering the field. The purpose of the OHHG Project is to preserve the documentary record of human genetics beginning with the period from 1950-2000. The OHHG Project is a collaboration of UCLA, the American College of Medical Genetics and the American Society of Human Genetics. We have begun to document the life stories of pioneers and leaders in the field using the formal tools of the oral historian, including digital video recording and printed transcripts. Dr. Victor McKusick assumed responsibility for the Moore Clinic on July 1, 1957, has disciplined information gathering through the compendium, *Mendelian Inheritance in Man*, and was present in the White House for the announcement of the completion of the Human Genome Project. We will use an interview with Dr. Kusick and a discussion of his early work on Peutz-Jeghers syndrome to illustrate how oral history analysis can add to our knowledge of the development of a discipline. The OHHG Project will create a resource that will help illumine the stories of human and medical genetics for geneticists, historians and investigators from all fields.

Phenotypic description of Hereditary Sensory Neuropathy Type 1A (HSN 1A) in two kindreds segregating the same mutation in the gene encoding Serine Palmitoyl Transferase Long Chain Subunit 1 (SPTLC1). M.P.

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Hereditary Sensory Neuropathies (HSN) are genetically and clinically heterogeneous, with a variable degree of sensory or pain loss. Classification is based on mode of inheritance and clinical features. We report a detailed phenotypic description of HSN 1A in two families segregating the same mutation in the gene encoding the long chain subunit of serine palmitoyl transferase (*SPTLC1*). A point mutation, 399T®G is the most common HSN 1A mutation to date, found in families of English extraction from the UK, Australia and Canada. The two large families described here descend from a small coastline stretch of Newfoundland, Canada. Both families were traced to Southwest England, suggesting a founder effect. Participants agreed to mutation testing and a neurological and electrophysiological examination. Sural nerve biopsies were done in six affected individuals. Clinical findings were consistent, but of variable severity and onset. Most women noticed first symptoms in the second or third decade and pregnancy appeared to exacerbate disease. Examination revealed signs of an age-related and length-dependent small fibre sensory neuropathy with associated pain and trophic skin changes but few signs of autonomic dysfunction. Motor involvement was common, with early wasting of thenar eminence and intrinsic hand muscles, and with accentuated distal leg muscle wasting resulting in *pes cavus* and foot drop. Primary axonal degeneration was confirmed in the electrophysiological and pathological examinations. This study represents an advance in the potential for genetic counselling and disease management.

Consumer organizations serving individuals and families with genetically-related conditions: potential lessons for outreach and education. *R.M. Fineman¹, C.A. Roeber², B.P. Oles³, J.P.N. Massad², C.E. Crain², N. Tashima².* 1) RMF Associates, Seattle, WA; 2) LTG Associates, Inc, Takoma Park, MD; 3) Human Ecology Dept, Rutgers Univ.

It is widely acknowledged that public and private health care and social services professionals, consumers, and policymakers are frequently uninformed about issues related to genetics, health, and health care. As a result, consumers and providers often are unable to make informed decisions about genetic health care issues. Research on consumer organizations that serve individuals and families with genetically-related conditions can provide valuable lessons for the development of strategies used to educate the audiences noted above. Forty-two consumer organizations that provide education and support to individuals and families affected by genetically-related conditions were selected and contacted for this research. The organizations were selected to allow the research team to identify: how the organizations vary in size, locale, structure, and mission; what roles the organizations play within systems of care; the range and direction of organizational activities in light of advances in human genomics research; outreach and education activities determined to be most effective; and barriers and facilitators encountered when conducting outreach and education (with a specific emphasis on ethnic, minority and under-served populations). Our research yielded several important themes related to the nature and scope and the successes and challenges consumer organizations have experienced including: development of message content; determination of communication channels; and audience targeting. We hope that the findings of this study will help inform outreach and education efforts by public and private health care agencies, and consumer organizations, in order to enhance informed decision-making for the individuals and families they serve.

Assessing the value of an expanded clinical genetics curriculum for medical students. *L.A. Demmer¹, M.M. Carlin², S.V. Barrett², U. Purwono², J. Jonassen², M.P. Pugnair².* 1) Pediatrics, Univ. Mass Medical School, Worcester, MA; 2) Office of Medical Education, Univ. Mass Medical School, Worcester, MA.

Purpose: In 1995, an enhanced genetics curriculum with a clinical focus was introduced into a year one medical student genetics course. New components included patient and family interviews, small group discussions, psychosocial and ethical concerns, role play, and letter-writing exercises to families. A complementary program was introduced in spring 2000 as a third year two-day genetics interclerkship, with opportunities to interview families, engage in panel discussions, and attend lectures on genetic technology. This study assessed the value of the interventions by comparing cross-sectional data for four groups of medical students.

Methods: Effect of the interventions was examined using AAMC Graduation Questionnaire data. Ratings of instruction time of four graduating classes were compiled: one with prior curriculum (GY 1998), two with new curriculum (GY 1999, GY 2000), and one with new curriculum plus interclerkship (GY 2001). Proportions of 'appropriate' ratings were compared.

Results: The 'appropriate' ratings of Genetic Counseling instruction time for GY 1998-2001 were 51.9%, 74.7%, 78.8%, and 90.3% respectively (N=395; 100% response rate). Comparison of proportions revealed a significant difference between prior curriculum (GY 1998) and new curriculum (GY 1999, GY 2000) ($p < .05$). The proportion of 'appropriate' ratings of the new curriculum plus interclerkship (GY 2001) was significantly higher than the prior curriculum and significantly higher than the cohorts with the new curriculum but no interclerkship ($p < .05$).

Conclusions: This study provides evidence of the value of clinical emphasis on a genetics curriculum, first implemented in preclinical work and later expanded upon in clinical years. This suggests that such 'longitudinal' curricular interventions can be successful in promoting an enhanced education experience as perceived by students.

The use of standardized patients enhances medical student learning in medical genetics. *A. Greb, A. Trepanier, M. Kavanagh, G. Feldman.* Ctr Molec Medicine & Genetics, Wayne State Univ, Detroit, MI.

To evaluate the effectiveness of utilizing standardized patients (SPs) compared to student role-playing in teaching medical genetics, first year medical students in our genetics course were randomly assigned one of two case studies. The first case (DMD) involved a pregnant woman at risk for carrying a dystrophin mutation. For this case, SPs were trained to present the clinical scenario. The second case (NTD) involved a 43-year old woman with a family history of spina bifida. For this case, students were given appropriate history information and instructed to play the role of the patient. For both cases, students were expected to ascertain the patient's concerns, review laboratory results, collect relevant history information, perform a risk assessment, explain the genetics and natural history of the condition, offer genetic testing and disclose results. The faculty facilitators in each group documented if students attempted each of these tasks. Data was collected from 8 of 11 facilitators. There were no significant differences with regard to tasks completed between the SP and the student role-play cases. Students were asked to complete an evaluation form to assess their perception of the usefulness of SPs compared to student role-playing (n=142). Sixty-eight percent of the students stated that the use of an SP enhanced their learning compared to student role-playing; 67% stated that the utilization of the SP was a beneficial addition to the DMD case; and 61% stated that they would recommend using SPs in the case studies again. Student comfort level when talking with the patient was also assessed. Those who participated in the SP case were more likely to report that they felt uncomfortable when talking to the patient (22%) than students who participated in the other case (13%). Many students commented that they took the encounter more seriously when an SP was the patient. In conclusion, although SPs and student role-playing appear to be effective tools for medical students to gain experience applying basic genetic principles in a clinical manner, we found that students preferred the SP encounter, which enhanced their learning experience.

Longitudinal Evaluation of an Educational Intervention - Prenatal Diagnosis and General Practitioners. S.A. Metcalfe^{1,2}, A. Flouris¹, M. Seipolt³, MA. Aitken¹. 1) Genetics Education Unit, MCRI, Royal Children's Hospital, Parkville, Vic, Australia; 2) Dept Paediatrics, University of Melbourne, Parkville, Vic, Australia; 3) Genetic Health Services Victoria, Royal Melbourne Hospital, Parkville, Vic, Australia.

Increasingly patients present to general practitioners (GPs) with genetics-related issues. Many studies worldwide have revealed that GPs have insufficient knowledge about genetics and genetics resources. Australian GPs believed their knowledge was deficient, recognised the need for further education and identified prenatal diagnosis (PND) as a priority area.¹ A study was designed to assess GPs' knowledge and practice regarding PND, and to evaluate the effectiveness of a case-based workshop. Invitations for the workshop were mailed out to >1000 antenatal shared-care GPs. The workshop was offered at 3 different hospital-based settings. Places were limited and 106 GPs in total attended. GPs completed a pre-questionnaire prior to attending, which included questions covering a range of genetics topics. GPs who attended the workshop were asked to complete a second (post-) questionnaire, which contained some questions identical to the first relating to PND and the workshop itself. GPs who completed both (94) were given knowledge scores for each questionnaire, which were used to evaluate the effectiveness of the workshop. Six-eight months after the workshop, follow-up surveys were again sent out to participants to investigate if they retained the knowledge learnt from the workshop. Immediately following the workshop, mean knowledge scores increased for about 75% of GPs. The main areas that GPs improved in knowledge were (1) distinction between screening vs diagnostic tests, (2) which women should be offered prenatal screening, and (3) the age-related risk of Down syndrome for younger women. The follow-up survey showed that, whilst knowledge levels did decrease over time, they were still significantly higher than prior to the education intervention, indicating long term retention of knowledge. 1. Metcalfe S et al, Needs assessment study of genetics education for general practitioners in Australia. *Genet Med*, 4:71-77, 2002.

Predictors of interest in hereditary cancer risk information among Cancer Genetic Network (CGN) registry participants. *S.J. Olson¹, A. Ziogas², L. Wenzel² and Cancer Genetics Network, Educational Working Group.* 1) School of Nursing, Johns Hopkins University, Baltimore, MD; 2) College of Medicine, Epidemiology Division, University of California, Irvine.

Individuals need a functional understanding of genetics to make informed decisions about participating in genetic counseling and/or testing, as well as adopting behaviors that may reduce cancer risk. While professional societies acknowledge the importance of education for informed decision-making, little is known of informational needs of those participating in cancer genetics registries. Therefore we examined predictors of interest in further information about hereditary cancer risk among participants voluntarily recruited to the CGN. Of 13,004 individuals belonging to 11,447 families who completed the CGN Core Questionnaire, 83% wanted additional genetic information. Significant differences in interest emerged by gender, marital status and age of the respondent. As anticipated, past participants in genetic testing and genetic counseling were significantly more interested in additional information. Multivariate analysis using generalized estimating equations that account for familial correlations, recruitment center, ascertainment mode, genetic testing- and counseling found that the strongest predictors of interest in further information were: gender, (females expressed more interest than males, OR 1.2, 95% CI 1.1-1.3), age of respondent (participants <40 yrs expressed more interest compared to those >70 yrs (OR 2.5; 95% CI 2.1-3.1), marital status, and history of cancer in first degree relatives (FDRs). Information interest increased with strength of family history, with 76%, 84%, 86%, 88% and 89% desiring information among those with 0, 1, 2, 3, or >4 family members with cancer respectively. Males were more likely to be interested when prostate or colorectal but not breast or ovarian cancer was present in their FDRs. However, females wanted more information when breast, colorectal, ovary, or prostate cancer was present in FDRs. Personal history of cancer, education, and race/ethnicity were not significant predictors. This information will guide future educational efforts within the CGN.

Program Nr: 1005 from 2002 ASHG Annual Meeting

Clinical applications of molecular genetics: An on-line course. *T. Namm.* Dept Health & Clinical Sci, Univ Massachusetts Lowell, Lowell, MA.

The June, 2000 announcement of the completed first draft of the human genome project immediately accelerated activity among pharmaceutical companies, genetics research venues, and medical geneticists to streamline molecular genetic protocols for the diagnosis, treatment, management, and cure of human genetic disorders. Because information generated by molecular genetic technology extends to all corners of health and medicine, keeping current with developments in these areas becomes a must, not only for the "traditional" student, but for those already employed in associated professions. For many years, web-based education has been, and will continue to be, the most efficient way for any student to keep current with the latest developments in any field, no matter where they are located, and no matter where the course is offered. I have developed an on-line course entitled "Clinical Applications of Molecular Genetics." Topics include laboratory techniques employed in molecular diagnostics; methodologies for sequencing the human genome; the diagnosis, treatment, and prevention of diseases using gene therapy; current cloning technology and its applications; stem cell research and its implications for medicine; the development of designer drugs based on the genetic basis of disease; cancer cytogenetics; microarray chips and gene expression; and genomics, including physiological genomics, pharmacogenomics, and developmental genomics. Ethical, legal, and social implications generated by these issues are also discussed. The course culminates with a discussion of the human proteome project, including goals and objectives, existing and future sequencing technology, and the storage of data utilizing complex bioinformatic databases. The poster outlines techniques utilized to teach modern molecular genetics in an on-line platform, citing especially those modifications that are necessary for teaching in the absence of the traditional classroom setting. Also included are samples of: "lecture notes", student discussion board postings, graphic integration techniques, chat line histories, and accessibility to related links. Course requirements, test administration procedures, and the student evaluation system are presented.

A pilot study on psychiatric genetic counseling: counselors needs. *H.L. Peay, J.D. McInerney.* National Coalition for Health Professional Education in Genetics (NCHPEG), Lutherville, MD.

NCHPEG is producing a CD-ROM, funded by the U.S. Department of Energy, to educate genetic counselors about psychiatric genetics. To guide development we surveyed two groups of counselors about their perceived needs and comfort. Few such data exist in the literature. While both groups were small and self-selected, and may not reflect NSGC membership as a whole, the results provide insights into counselors' knowledge of, and comfort with, psychiatric genetics. In the first phase, 209 NSGC members completed an on-line survey. Counselors identified a need for information on: schizophrenia and affective disorders; major psychosocial issues; evaluating psychiatric diagnoses; the etiology of psychiatric disorders; risk assessment; evaluating multiplex families and genetic subtypes; managing uncertainty; teratogenicity of psychotropic medications; and counseling for those at risk. During field-testing 41 practicing genetic counselors (PGC) and 28 second-year genetic counseling students (GCS) completed a pre-test survey. When asked how prepared they feel to raise the issue of psychiatric disease with their clients, 32% (PGC) and 67% (GCS) felt either very unprepared or somewhat unprepared; when asked how prepared they feel to answer questions about psychiatric disease from their clients, 44% (PGC) and 96% (GCS) felt either very unprepared or somewhat unprepared; when asked how comfortable they feel providing psychosocial counseling for psychiatric disease, 54% (PGC) and 78% (GCS) felt either very uncomfortable or somewhat uncomfortable. When asked how often they raise issues related to psychiatric disease when they take a family history, 22% (PGC) and 46% (GCS) responded never or seldom. When asked what limits their ability to provide psychiatric genetic counseling, 59% (PGC) and 93% (GCS) chose "My understanding of psychiatric diagnoses," 83% (PGC) and 82% (GCS) chose "My knowledge of research in psychiatric genetics," and 39% (PGC) and 75% (GCS) chose "My lack of knowledge/experience with psychosocial issues involved in psychiatric genetic counseling." These results suggest a need for education on psychiatric genetics within the genetic counseling community.

Crafting a custom cancer genetics web site for primary care physicians. *S.L. Tobin¹, C.D. Jacobs², J.M. Ford², A. Boughton³, K. Ritter², D. Bamber² and Education Task Force.* 1) Ctr Biomedical Ethics, Stanford University, Palo Alto, CA; 2) Cancer Genetics Clinic, Stanford University, Stanford, CA; 3) Twisted Ladder Media, San Francisco, CA.

The use of genetic tests as tools to assess hereditary cancer risk is expanding rapidly. When such tests are used in the context of a clinical program that includes genetic counseling and consideration of a detailed family medical history, the results are often of substantial value in guiding surveillance and interventions. Access to specialized cancer risk assessment programs can be a key factor in quality patient care. Yet because the clinical applications of genetic tests have emerged so recently, a number of studies have documented the fact that many primary care physicians are unaware of or unsure about how to identify individuals or families who could benefit from specialized genetic services. The Stanford Program for Applied Cancer Genetics is funded by the California Department of Health Services, Cancer Research Section and includes a component to accomplish educational outreach to primary care physicians, especially those whose practices include substantial underserved populations. An Education Task Force was formed, and a workable approach to obtain direct feedback about the educational needs of primary care physicians was developed. Two thousand recruitment letters were sent to primary care physicians in the targeted geographic area. The prospective participants were asked whether they would be willing to evaluate the educational initiatives produced by the Applied Cancer Genetics Program, and their current use of educational resources and their preferences were assessed. The results from 131 responses suggested a demand for more computer-based resources. With the further advice of the Education Task Force, an evaluation version of a cancer genetics web site is under development. This web site utilizes a case-based approach and concentrates on the knowledge and skills that are relevant for appropriate identification, intervention, and referral of patients at risk for hereditary non-polyposis colorectal cancer (HNPCC). The prototype version of this site will be demonstrated.

Web-Based Survey of Families with Autism: Reassessing the Need for Genetic Education. *C.M. Wolpert¹, B. Rosen-Sheidly², S.L. Donnelly¹, L.A. Elston¹, C.S. Turner¹, S.E. Folstein², M.A. Pericak-Vance¹.* 1) Duke Univ Medical Ctr, Durham, NC; 2) New England Medical Ctr, Boston, MA.

Previously we reported the results of a pilot survey mailed to families participating in an autism genetic research study. Data indicated that education of the public and health professionals about genetic research findings and their potential clinical application has lagged. ExploringAutism.org, a web site dedicated to helping families with autism stay informed about autism genetics, was developed to provide a credible source of understandable genetic information. Because the population in the pilot study was limited to a small number of families participating in genetics research, we hypothesized that responses would differ in a larger, non-research oriented population. An online version of the survey was included on the web site. Since February 2002, over 9500 people have visited the web site; 433 submitted surveys (a response rate of 6%). Similar to the pilot population, 387 (89%) of respondents have a family member with autism. In contrast to the pilot data, a majority of respondents (93%) indicated that they are not participating in research. When asked about possible causes of autism, 86% cited genetic factors, while 33% cited environmental factors; and 31% cited vaccinations (despite lack of medical evidence for the latter). 24% of respondents correctly identified the recurrence risk for autism, but 39% percent grossly overestimated the risk and 31% were unsure. Most respondents favored both genetic testing to confirm a diagnosis of autism (92%) and prenatal diagnosis of autism (75%). 98% indicated an interest in learning more about the genetic basis of autism; 98% felt that this web site was informative. 70% felt that learning about the genetic basis of autism reduced their anxiety. These results suggest the widespread need and desire for education regarding the genetics of autism are not limited to families participating in genetic research. Additionally, the data indicate that web-based tools are an effective method to reach these families.

Knowledge and attitude towards ethical issues in human stem cell research: a survey among medical staffs in Taiwan. *SJ. Lin^{1, 2}, CH. Chang³, PL. Kuo³, SC. Hu⁴, MC. Huang⁵, YH. Wang⁵.* 1) Department of Pediatrics, National Cheng Kung University Hospital; 2) Bureau of Health Promotion, Department of Health, Taiwan; 3) Department of Obstetrics and Gynecology, Cheng Kung University Hospital; 4) Department of Public Health, Medical College, Cheng Kung University; 5) Department of Nursing, National Cheng Kung University, Tainan, Taiwan.

Human stem cell research has drawn important attention worldwide. The National Medical Ethics Advisory Committee in the Health Department of Taiwan government had proposed their recommendation and regulation after several public audit in February 2002. The regulation permits researchers using materials from abortion and assisted reproduction, but prohibit nuclear transfer on human cells. In order to understand attitudes towards ethical issues in human stem cell research, we conducted a survey among medical students, obstetricians, pediatricians and neurologists. Self-administered structured questionnaire was used. The results analyzed were based on 61 physicians, included 28 pediatricians, 19 obstetricians and 14 neurologists. 53 medical students who finished their clerkships in the above clinical departments were also included. We found that the key question in the ethical debate on human stem cell research lies on the moral status of embryo/fetus. While 49% of physicians considered the minimal requirement for a human being depended upon the survival after delivery, so a embryo was not considered as a human; there were 45% of students considered human life begins at the moment of conception. However, there were no correlation to their religious belief. Above 50% of the respondents agreed to use any materials for stem cell research. Even for the somatic cell transfer that might lead to human cloning, there were 69%; of respondents agreed upon this technique. Among them, obstetricians had the highest acceptance rate (79%). However, above 50% of the respondents did not know any regulation or recommendation from government officials. In conclusion, physicians and medical students generally took optimistic views on human stem cell research. However, there exist significant controversy and confusion about the moral status of fetal/embryo.

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Preimplantation Genetic Diagnosis: attitudes and opinions. *E.S. Ramos, C.A. Vicente, M. Floria, A.K. Bartmann, A.R. Zambianchi, L. Martelli.* Dept Genetics, School of Medicine of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, Sao Paulo, Brazil.

Preimplantation Genetic Diagnosis (PGD) is a procedure derived from in vitro fertilization and prenatal diagnosis techniques that make it possible to detect some genetic disorders in embryos before they are transferred to the uterus. At the moment, there are no legal regulations in Brazil concerning PGD, but many hospitals are prepared for its clinical use and it has been performed in some services. In contrast, Brazilian law forbids the pregnancy termination in the majority of the cases. The objective of this study was to assess the health students/professionals' attitudes and preferences to embryo biopsy and prenatal diagnosis through Chorionic Villus Sampling (CVS)/Amniocentesis (AF). The design involved a structured questionnaire, which includes other related issues as religion, abortion, familial risk for genetic diseases, and the destination of not transferred embryos. The population sample (a total of 357 respondents) included students from the Medical and Nursing Schools of Ribeirao Preto - University of Sao Paulo and physicians/professors, physicians/residents, and other health professionals. Fifty eight percent were in favor of PGD, whereas 30% preferred diagnosis through CVS/AF, and 12% abstained. The main reason for the PGD choice was that it is performed during the earliest stages of development and abortions can be avoided. In this study, there was also the opportunity to promote the discussion, in special between the students, about ethical importance and social acceptability of new techniques. Supported by: CREMESP, FAEPA, FAPESP, CNPq, CAPES.

Mutation detection rates and organ system involvement in tuberous sclerosis complex. *A.J. Williams, M.W. Bishop, K.-S. Au, H. Northrup.* The University of Texas Medical School, Houston, TX.

Introduction: Tuberous sclerosis complex (TSC) is a dominant condition involving hamartoma and hamartia development in varied organ systems. Causative mutations are found in the *TSC1* and *TSC2* genes. Diagnoses are made using published criteria, with no requirement for involvement in >1 organ system. Mutation detection rates range from 68% to 78%. The presence of large gene deletions in *TSC2* or somatic mosaicism for the causative mutation may account for the remaining cases. **Hypothesis:** Mutation detection rates are lower in individuals with TSC and limited organ system involvement. **Methods:** Mutations in *TSC1* and *TSC2* were identified by direct sequencing of coding exons; results were entered into a Microsoft Excel Database and correlated with patient symptoms. Analysis of organ system involvement was conducted 1) by the number of systems affected (skin, brain, heart, eye, other) and 2) by the embryological derivation of the systems affected (ectodermal-skin, eye, brain; mesodermal-kidney, heart). **Results:** To date, 82 samples from persons with sporadic TSC have been sequenced with a detection rate of 77%. Of the 82 samples, 25 came from persons with 2 affected organ systems, while 57 came from those with 3+ affected systems. Among those with 2 systems affected, 16 were mutation positive (64%) compared to a 82% positive rate in the group with greater involvement (47/57). An evaluation of mutation detection rates by embryological derivation of affected organs yields a 62% detection rate (13/21) in those with symptoms from one source, versus 82% (50/61) in those with symptoms from two. **Conclusion:** Mutation detection rates are less in a TSC population with limited organ system involvement, particularly when affected organs arise from one embryonic tissue. In cases where symptoms arose from one source, the derivation was from ectodermal tissue; none had symptoms solely in organs of mesodermal origin. Mesoderm gives rise to hematologic tissues from which DNA is extracted for most genetic testing. Our findings suggest that somatic mosaicism arising from a post-zygotic mutation in ectodermal tissue may play a role in the ability to identify TSC mutations.

Patient acceptance of amniocentesis after a positive serum AFP screen is not influenced by level of risk. *K.M. Borsack¹, A.B. Metzzenberg¹, S.W. Morton², J.D. Greenberg², R. Petrucha², B.J. Huffaker², J. Siegel-Bartel².* 1) California State University Northridge; 2) Southern California Kaiser Permanente, Los Angeles.

Development of rational public health policy depends on understanding and predicting patient utilization. We retrospectively examined self reported anthropologic factors influencing amniocentesis utilization in 132 patients screen positive (XAFP+) for Down syndrome(DS) from an urban clinic with ethnically varied population from 2001. In California, the state XAFP program reports risk level for DS as a numeric ratio with 1/190 as the cutoff. We found **amnio uptake did not vary with magnitude of DS risk**. Patients with DS risk of 1/20 or greater had 50% acceptance; 1/22-1/96 had 49% acceptance; 1/100-1/190 had 55% acceptance. Patients with XAFP+ who are 38+ years at EDC may have a XAFP risk less than their age related risk. Utilization in these older patients also did not depend on numeric level of risk; acceptance of amnio was 38% if the XAFP risk was less than the age-related risk and 25% if the XAFP risk was greater than the age-related risk. Factors which correlated to patient choice for amnio included: 1)no living children 73%, one LC 54%, 2+ LC 36%($p<0.05$); 2)no previous SAB 62%, one previous SAB 52%, 2+ previous SAB 18% ($p<0.05$). There was a trend for correlation 1) if planning to abort for an abnormality or unsure 87%, planning to continue despite an abnormality 12.5% ($p<0.2$); 2)not employed outside the home 70%, all employed women 48% ($p<0.1$); 3)ethnicity Caucasian,non-Latina 68%, Black 61%, Latina 48%, Asian 47% ($p<0.2$). Latina patients using an interpreter were more likely to accept amnio 55%(16A/13D)($p<0.2$). We postulate that presence of the interpreter alters the counseling dynamic; the interpreter may be accepted by the patient as a nonmedical patient advocate. **We are intrigued that the risk level did not influence patient utilization, and postulate that patients view their risk in a binary fashion, rather than as a numeric continuum.** Alternatives to numeric risk presentation (such as graphs) may help patients understand their risk, and should be evaluated.

Communication and Control in Families with a History of Breast and, or Ovarian Cancer

. R.H.

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This study explores how family communication patterns and family scripts influence the dissemination of genetic information and the sharing of feelings about genetic inheritance in families of healthy women who have attended a cancer genetics risk clinic because of their family history of breast and, or ovarian cancer. Family scripts are sets of expectations, beliefs, and norms that assign meaning to patterns of interaction, connect generations and provide guidance for action.

We conducted an IRB reviewed exploratory, qualitative study at a major clinical and research cancer center in the United Kingdom from January through June 2000. Twenty-one semi-structured, in depth interviews were conducted using a purposive sample of women coming to the cancer genetics risk clinic for the first times, supplemented by 5 months of participant observation.

We found several communication patterns: open and supportive; directly blocked, indirectly blocked, self-censored and use of third parties. These varied between members of one family and between families. Some family members shared their feelings and discussed ways of trying to avoid developing breast or ovarian cancer; for others disseminating information or just talking about inherited susceptibility for breast and, or ovarian cancer fell into the script violation category; still others tried to renegotiate their family scripts.

As cancer risk genetics is increasingly incorporated into health care delivery, understanding communication and dissemination of genetic information in families with a history of breast and, or ovarian cancer becomes even more essential.

Genetics & Your Practice Online: An Educational Genetic Resource Project. *T.M. Creeden¹, S.J. Durfy², R.M. Fineman³, M. Greenup¹, N. White⁴.* 1) March of Dimes, White Plains, NY; 2) Illuminata, Inc., Seattle, WA; 3) Robert Fineman Associates, Seattle, WA; 4) Full Circle Associates, Seattle, WA.

With funding from the Robert Wood Johnson Foundation, the March of Dimes Birth Defects Foundation is developing an interactive online genetics information resource and continuing education (CE) program. This resource will provide tools and guidance for health care and social service professionals to assist them in further integrating genetics into their patient services. This project provides a practical, resource-oriented web site to serve as a companion to existing March of Dimes genetic education curricula. The web site will be designed for primary care professionals (PCPs) and will be customized for three patient types (preconception/prenatal, infants/children, and adolescents/adults). Online tools specific to these patient types will be provided. The project goals are to help PCPs 1) Obtain and interpret family health and social history information; 2) Promote the use of tools and collateral resource materials; 3) Refer to genetic services as appropriate and necessary; 4) Identify local genetic resources; 5) Enhance their comfort in interacting with patients who have genetic health care issues. Health care professionals will be able to acquire information about locating genetic resources, genetic testing and screening, family health and social histories, communicating with patients on genetic issues, and clinical genetics research. In addition, users will be able to obtain CE credits online, plus information on other March of Dimes genetics education programs such as the Genetics & Your Practice CD-ROM and lecture series. In preparation for this new online genetic education resource, a needs assessment was performed involving online research, recommendations from professional organizations, expert interviews, an extensive literature review, surveys of health professionals, and a review of current genetic education programs. Based on this research, we established the nature and scope of this web site project. Thorough process and outcome evaluations of this project are planned. The web site is anticipated to be available to users by the end of 2002.

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Nurse Practitioners' interest in and recommendations for online genetics continuing education (CE): A survey of Southern California (CA) Nurse Practitioners (NPs). *S. Durfy*¹, *T. Creeden*², *B. Izykowski*³, *B. Bendure*², *R. Fineman*⁴. 1) Illuminata, Inc, Seattle, WA; 2) March of Dimes, White Plains, NY; 3) California State University, Long Beach, CA; 4) Robert Fineman Associates, Seattle, WA.

The March of Dimes Birth Defects Foundation, with funding from the Robert Wood Johnson Foundation, is developing an interactive online genetics information resource and CE program, Genetics & Your Practice Online, for primary health care and social services professionals. In developing this new online resource, an extensive needs assessment is being performed. We report here on one aspect of the needs assessment, i.e. a survey of 700 southern CA NPs. Respondents (n=245 certified NPs) practice in varied settings, encompass new and veteran NPs; approximately 3/4 have masters degrees. About 2/3 report no courses in genetics and most do not feel their educational background has prepared them to incorporate genetic advances into their NP practice. About 3/4 report they would find additional genetics education beneficial to their practice; 1/4 would not. In the preceding year, a majority of respondents had not sought information on genetic disorders or tests, and a majority reported they had not referred a patient for genetic services or been asked about genetics by their patients. For NPs the most important features in an online CE genetics course were that the authors be experts in the field, NPs have flexibility in selecting what they learned, the registration procedures not be time consuming, privacy be assured, links to general medical databases are provided, and the CE web site provide information of value to patients. The least important features were the ability to interact online with other professionals using the CE site and download items to personal digital assistants. NPs are an important target audience for genetic education efforts. These survey results underline the importance of understanding the perceptions and characteristics of the target audience and the attributes of their practice(s) when designing CE programs. Also, it is important to consider personal learning styles, customization and administrative issues in the design of online genetics CE programs.

Assessing Coping Strategies in Families with Jarcho-Levin Syndrome. *J. Acevedo¹, N. Ramirez-Lluch², A.S. Cornier³*. 1) Dept of Nursing, University of Puerto Rico at Arecibo, Arecibo, PR; 2) Department of Ortopedics, Mayaguez Medical Center, Mayaguez, P.R; 3) Division of Genetics, Ponce School of Medicine, Ponce, P.R.

Jarcho-Levin syndrome (MIM#277300) also known as Spondylothoracic Dysplasia syndrome(STD)is an autosomal recessive disorder with high prevalence in Puerto Rican population.The phenotype includes multiple anomalies of the ribs and vertebra in a short thorax, rigid and short neck and dwarfism. Familial understanding and subsequent management of this disorder represents a serious challenge to them. Identifion of patients and parents sucessful coping strategies provide the needed tool to improve patient health status and family adaptation to the illness process. This study described parental and patients coping strategies in dealing with JLS. The Coping Health Inventory for Parents and the Impact on Health Status Scale were used as research tools. Parental most useful coping strategies are: understanding the health care situation through communication with other parents and consultation with health care team; family integration, cooperation, and optimistic definition of the situation; and mantaining social support, self esteem and psychological stability. Patients indicated that being treated as a normal child and support of their parents were helpful in the adaptation process. Contradictory findings were obtained on the level of parental interference in their lives. Some degree of social isolation as a manifestation of clinical depression was noted in 11% of patients. The results shown that there is need to consider parental and patient's reactions to the disorder. Successful coping should be assess and teach to family members. Specific attention should be given to patient's interpretation of parental support and its effectiveness. Patients should be encouraged to openly express their feelings especially toward parental interference in their life.

Patient vs. physician as the target of educational outreach about screening for an inherited susceptibility to colorectal cancer: a randomized controlled trial. S. Loader, C. Shields, J.C. Levenkron, P.T. Rowley. Univ Rochester, Rochester, NY.

Will more patients seek genetic services if a tertiary medical center mails them relevant information in layman's terms or sends the same information in professional language to their physicians? We hypothesized that educating the patient directly would result in a larger proportion of patients receiving genetic services because the patient might feel a greater responsibility to alert relatives than would their physician and because the physician might be reluctant to raise genetic issues because of unfamiliarity with cancer genetics. We identified patients with colorectal cancer at <60 y from 1987-1999 in a 5-county area including Rochester. Qualifying patients (those reporting having at least one 1° or 2° relative with colon cancer) were randomized to either the patient-education group or the physician-education group. Patients in the patient-education group received a brochure in lay language describing inherited susceptibility to colorectal cancer, an invitation to come for genetic counseling, and an offer of DNA testing. In the physician-education group, the patient's physician was sent information in professional language and invited to refer the patient for genetic counseling and the offer of DNA testing. Both groups were offered services free of charge. In the patient-education group (51), 21 (41%) came for counseling and 16 (31%) chose testing. In the physician-education group (52), 26 (50%) came for counseling and 20 (38%) chose testing. These two educational approaches did not significantly differ in percentages of patients receiving counseling or choosing DNA testing. We hypothesize that patients receiving a letter from their personal physician about the availability of genetic counseling regarded it as a *recommendation* to seek genetic counseling and this may have counterbalanced the advantage we had hypothesized for educating patients directly. No patient in either group had been referred for genetic counseling by his/her physician spontaneously, suggesting that tertiary centers should consider making efforts at such educational outreach. (CDC UR6/CCU217194).

The Role of Genetics Professionals in Susceptibility Testing: Lessons from Factor V Leiden. *E.A. Hellmann¹, S. Moll², N.D. Leslie³*. 1) College Allied Health Sciences, University of Cincinnati, Cincinnati, OH; 2) Department of Hematology/Oncology, University of North Carolina, Chapel Hill, NC; 3) Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

Genetic testing for factor V Leiden, the most common inherited risk factor predisposing to venous thrombosis, was widely incorporated into clinical practice before consensus guidelines regarding the clinical implications were available. This study examines knowledge, satisfaction with information received from health care providers, health perception, and opinions of genetic testing of 110 individuals who tested positive for factor V Leiden between 1995 and 2001 to assess their experience of genetic susceptibility testing at a time of clinical uncertainty. Surprisingly, fourteen individuals (13%) did not recall being informed of test results and 39% did not recall giving informed consent for testing. Reportedly, results were given by a genetics professional in only 4% of cases. The magnitude of thrombosis risk associated with factor V Leiden was incorrectly estimated by 79% of participants. Patients were dissatisfied with the information they received about factor V Leiden; 64% felt they were not given much information and only 47% felt that their health care providers had an understanding of the condition. A majority (88%) were glad to know their genetic status. Most genetic testing for factor V Leiden is offered outside the auspices of a genetics clinic. To optimize patient understanding of the testing process and the implications of test results, strategies and materials need to be developed for use in the primary care setting. It is likely that such strategies will have application to the anticipated expansion of genetic predisposition testing through primary care oriented practices.

Enhancing genetics education in medical school: An approach using a web-based course program. *S. Taylor¹, J. MacKenzie², K. Harrison¹, I. Sumargo¹, M. Fleming³, E. Van Melle⁴.* 1) Dept Pathology, Queen's Univ, Kingston, ON, Canada; 2) Dept Pediatrics, Queen's Univ, Kingston, ON, Canada; 3) Information Technology Services, Queen's Univ, Kingston, ON, Canada; 4) Faculty of Education, Queen's Univ, Kingston, ON, Canada.

The increasing use of genetic information in clinical practice has created challenges for educators to identify effective strategies for undergraduate medical students to learn, retain and apply genetics concepts. Further, future physicians need skills to keep abreast of advances in the field and refer appropriately. Within the condensed medical school curriculum at Queen's University, challenges include the volume of material, and a condensed timeframe. A project was initiated to evaluate the potential of using a web-based course program (Web CT) to organize and improve accessibility to course material and create an opportunity for students to apply these concepts in the evaluation and management of a clinical case. Members of the team provided expertise in genetics, education and information technology. The team adopted the "ICE" Model (Ideas, Connections and Extensions), a theory that defines learning as a multistage process and encourages the development of higher order thinking skills. In addition to the use of the web site to provide access to reorganized course material, students were divided into small groups and required to review a case study posted on the site, and to email the instructors with a request for a genetic test and the rationale. Once requests were received, the test results were posted on Web CT. The groups were required to present an evaluation of their case and the implications of the genetic test results to their peers. 93% of students indicated that the web-based technology was easy, or very easy, to navigate demonstrating enhanced access to course material. 92% of the students reported active or very active participation in discussions around the clinical case studies. These results demonstrate an approach that captures the potential for computer technology to facilitate student learning in the field of medical genetics.

The Frequency and Characterization of Mitochondrial Hearing Loss in a National DNA Repository from Deaf US Probands. *A. Pandya*¹, *A. Massiello*¹, *B. Landa*¹, *X.J. Xia*¹, *K.O. Welch*², *K.S. Arnos*², *S.H. Blanton*¹, *W.E. Nance*¹, *G. VanTuyle*¹. 1) Medical College Virginia at VCU, Richmond, VA; 2) Gallaudet University, Washington DC.

Mitochondrial mutations are now recognized as an important cause of genetic hearing loss. The A1555G substitution in the 12SrRNA gene is often associated with sensitivity to aminoglycosides. Substitutions at positions 7443, 7444, or 7445 located in the cytochrome oxidase I gene (COX I) stop codon, and adjacent to the 3' end of tRNA ser (UCN) are also associated with non syndromic hearing loss (NSHL). Previous studies in deaf probands from Mongolia, China and Spain have revealed a high incidence between 10% and 30% for the A1555G substitution. The mutations at 7445-7443 position are heterogenous and occur with a frequency of 1% in Mongolia. The high frequency of the A1555G mutation and the potential for preventive intervention makes this gene an attractive candidate for newborn screening. To determine the frequency of these mutations in the United States we screened a national DNA repository of 372 deaf probands who were ascertained through a nation-wide survey of educational programs for the deaf, conducted by the Gallaudet Research Institute. The mitochondrial mutations were detected by PCR-RFLP and confirmed with DNA sequencing. The frequency of the 7445-7443 substitutions were about 1% , similar to our finding in Mongolia. The mutations in the US probands, however, included the A7445G and the G7444A substitutions which differs from the spectrum of mutations observed in deaf Mongolians. Surprisingly, we did not find a single proband with the A1555G substitution. We are currently evaluating cell lines from affected individuals to clarify the pathogenic mechanisms involved in the 7444 and 7445 mutations. Further studies to determine the frequency of the A1555G substitution in the general population will be necessary before assessing the potential value of newborn screening for either of the mitochondrial mutations.

ASSOCIATION OF GENETIC POLIMORPHYSM APOB (VNTR) IN PATIENTS WITH ISQUEMIC CARDIOMYOPATHY OF MEXICAN POPULATION. *M.P. Gallegos Sr¹, H. Rangel², M. Zuniga³, L. Arnaud¹, L. Lopez⁴, L.E. Figuera⁵, T.J. Beltran⁶, M.R. Arechavaleta⁶, L. Sandoval⁵, G.M. Zuniga¹ and heart disease.* 1) Medicina Molec, CIBO IMSS, Guadalajara Jalis, Guadalajara, Mexico; 2) Ciencias de la Salud, Universidad de Guadalajara, La Cienega, Ocotlan,Jal. Mexico; 3) Unidad de Terapia, Cardiologia,; 4) Laboratorio de Lipidos,; 5) Genetica, CIBO,; 6) Departamento de Endocrinologia,HE,CMNO,IMSS.

The coronary heart disease to produce high rates of mortality in many countries of world. Epidemiological studies have established a positive relationship of coronary heart disease and low density lipoprotein cholesterol with apoB levels. The 3 flanking region of the apoB gene contains a hypervariable region consisting of variable number of tandemly repeated short AT rich DNA sequences (VNTR). We determinate the association and frequency of apoB VNTR polymorphism in patients with isquemic cardiomyopathy (IC) of Mexican population. Were analyzed the polymorphism VNTR apoB in 50 controls and 131 patients with IC. The analysis statistical of polymorphism apoB VNTR showed a significant association with allele 39, 1% in controls and 35.49% in patients ($p>0.05$). These results showed that individuals with allele 39 VNTR apoB were associated with IC in Mexican populations.

Interleukin-1 polymorphism significantly associated with increased risk for hip osteoarthritis in females. I.

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Osteoarthritis (OA) is characterized by degradation of articular cartilage and formation of new bone in which both genetic and mechanical factors play a significant role. Identification of genes that influence disease susceptibility may give insight which pathways may be involved in the onset of OA. The interleukin-1 (IL1) gene cluster on chromosome 2q contains 3 related genes within a 430 kb region, IL-1A, IL-1B and IL1RN. These genes encode the pro-inflammatory cytokines IL-1a and IL-1b and their endogenous receptor antagonist IL-1ra, respectively. Especially IL-1b is involved in modulating the chondrocyte metabolism by stimulating the chondrocyte to produce metalloproteinases (MMPs). MMPs degrade extracellular matrix components in articular cartilage. The rare -511T allele in the promoter of the IL1B gene was found to alter a HC5 promoter element and to be associated with increased secretion of IL-1B after LPS stimulation (1). In this paper the IL1B C-511T polymorphism was studied by association with the occurrence of radiographic OA (ROA) in knees, hips, hands, and spine in a random sample of 55-65 year old subjects from a population-based cohort (The Rotterdam study, N = 744). A dose response association with hip ROA was observed for hetero- and homozygous carriers of the -511T allele (crude OR = 1.67, 95% CI 0.94-2.96 and 2.56, 95% CI 1.15-5.43, respectively). The effect appeared significantly high in female carriers (crude OR = 4.18, 95% CI 1.38-12.64 and 7.62, 95% CI 2.13-17.24, respectively) and not present in males (crude OR = 1.14, 95% CI 0.53-2.45 and 1.29, 95% CI 0.46-3.62, respectively). These data indicate that the C-511T polymorphism in the IL1B gene is associated with increased secretion of IL-1b after LPS stimulation and may contribute to the onset of hip OA in females. 1) Seymour et al. ASHG 2002 abstract.

Does apolipoprotein E genotype influence the progression of multiple sclerosis? *R. Cittadella¹, V. Andreoli¹, I. Manna¹, A. La Russa¹, P. Serra¹, G. Savettieri², G. Salemi², S. Bonavita³, C. Caltagirone⁴, U. Nocentini⁴, M.C. Fazio⁵, P. Girlanda⁵, F. Le Pira⁶, A. Reggio⁶, M. Liguori⁷, G. Logroscino⁸, A. Lugaresi⁹, L. Toma⁹, P. Valentino¹, A. Quattrone¹.* 1) Inst. of Neurological Sciences, National Research Council, Pianolago, Cosenza, Italy; 2) Institute of Neuropsychiatry, University of Palermo, Italy; 3) Department of Neurological Sciences, II University of Napoli, Italy; 4) I.R.C.C.S. Fondazione Santa Lucia, University of Roma Tor Vergata, Italy; 5) Department of Neurosciences, Psychiatry and Anaesthesiology, University of Messina, Italy; 6) Department of Neurological Sciences, University of Catania, Italy; 7) Department of Neurological and Psychiatric Sciences, University of Bari, Italy; 8) Department of Neurology, Ospedale Miulli, Bari, Italy; 9) Department of Oncology and Neuroscience, University of Chieti Gabriele d'Annunzio, Italy.

To investigate the association between apolipoprotein E (APOE) polymorphisms and the progression of multiple sclerosis (MS) we investigated 428 subjects affected by clinically defined MS, with a disease duration of at least three years. We collected data concerning the age at onset of MS, clinical type, disease duration and disability according to the expanded disability status scale (EDSS). We also calculated the progression index (PI) to evaluate disease progression. APOE genotyping and the —491 A/T polymorphisms of the APOE promoter were determined. The PI was associated directly with age at onset and inversely with the relapsing remitting form of MS. Carriers of the e2 allele showed a lower PI, whereas no association was observed between the APOE e4 allele and clinical characteristics of our study population. We also investigated the —491 A/T APOE promoter polymorphisms in 236 MS subjects and did not find any association between the —491 A/T polymorphisms and selected clinical variables. Our findings indicate that in our population the APOE e2 allele is inversely associated with the PI, suggesting a protective role of this allele in MS progression. The APOE e4 allele and the —491 A/T APOE promoter polymorphism were not associated with a more rapid course of MS.

A NOS3 intronic polymorphism as a risk factor in ischemic stroke. *a.c. Dutra*. Neuroscience, JFK Medical Center, Edison, NJ.

Stroke is the leading cause of disability and the third leading cause of death in the western hemisphere. In the United States approximately 80% of all stroke patients suffer from ischemic infarction caused by thrombosis or embolism. The endothelial nitric oxide synthase gene (NOS3) has been the subject of recent research as a candidate gene contributing to the development of ischemic stroke. The product of this gene, the enzyme nitric oxide synthase, is the only generator of the free radical nitric oxide, in the vascular endothelium, which mediates endothelium-dependent vasodilatation and has also antithrombotic action. There are 2 common polymorphisms in intron 4 of NOS3 gene which have been described, consisting of a 27-bp consensus sequence repeated 4 times (allele a) or five times (allele b). A study in a Chinese population implicates the allele a as a risk factor for ischemic stroke. Our study proposes to analyze the association between this 27-bp polymorphism, and ischemic stroke in an ethnically heterogeneous population in central New Jersey, United States. We studied 400 consecutive patients presenting with ischemic stroke or TIA to a stroke unit of the hospital JFK Medical Center in central New Jersey. Clinical data and brain imaging studies (CT and /or MRI) were collected for all patients. A control dataset was also generated of individuals without stroke matched for age and risk factors recruited from the same region as the stroke population. The genotype of the polymorphism was determined by polymerase chain reaction (PCR) using the forward primer: 5' AGGCCCTATGGTAGTGCCTTT 3' and the reverse primer: 5' TCTCTTAGTGCTGTGGTCAG 3'. The alleles a (393-bp PCR product) and b (420-bp PCR product) were separated by gel electrophoresis in a 6% acryl amide gel. We found a significant difference in the frequency of the ab versus bb genotype between stroke patients and controls (74% versus 61%; $P=0.014$). The genotype aa was rare with frequency below 2% in the studied population compared with 6.5% in the Chinese population. Our results support and extend those of a previous study on Chinese patients on the potential role of the allele a of the NOS3 gene as an independent risk factor for ischemic stroke.

Frequency of Fragile X in Multiplex Autism: Testing the AGRE families. *W.T. Brown¹, S.L. NOLIN¹, C.S. DOBKIN¹, G.S. HOUCK¹, A. GLICKSMAN¹, X.D. DING¹, A.D. GARGANO¹, L. CRAWFORD², N. GITCHO², S.J. SPENCE², D.H. GESCHWIND², AGRE^{1,2}.* 1) Dept Human Genetics, NYS Inst Basic Research, Staten Island, NY; 2) Program in Behavior Genetics, Neuropsychiatric Institute and Neurology Dept, UCLA School of Medicine, Los Angeles, CA.

Autism is behaviorally characterized by profound deficits in social interaction, delayed and deviant communication, and unusual patterns of interests. It has high heritability. The biological basis of autism is unknown, but several genetic syndromes have been associated with it, including the fragile X syndrome. The Autism Genetic Resource Exchange (AGRE) is a publicly available resource of well-characterized multiplex families for genetic studies of autism. AGRE families were recruited without regard to prior testing but families with known neurogenetic syndromes were excluded. In order to better characterize this resource and to provide more information on the association with fragile X, we conducted fragile X DNA analysis (Brown 1993) on one proband in each of 480 AGRE families, with follow-up family studies when indicated. Testing revealed six families to be positive for fragile X. Review of a sampling of 29 chart records showed 11 (38%) had had prior negative genetic testing. Thus, the prevalence of fragile X among the approximately 298 previously untested AGRE families was estimated to be 2.0%. Previous prevalence studies of fragile X in autistic samples have reported frequencies ranging from 0 to 16%; with a mean of around 4%; (Feinstein 1998). The variations may be due to sample sizes, ascertainment differences or differing methodologies. Our frequency of 2.0% is similar to a report of 1.6% among 123 unrelated autistic individuals (Bailey 1993), but lower than the 13% we found on an earlier multicenter study of 183 individuals (Brown 1986). We suggest a growing awareness of fragile X syndrome would increase the probability of prior fragile X screening in multiplex autism families which would have excluded their inclusion among the AGRE families. The observed frequency of 2% is similar to the overall mean of 4% observed in other studies and indicates a significant association of fragile X and autism.

Association of the C677T and A1298C polymorphisms in the MTHFR gene with migraine risk. *I. Kara¹, A. Sazci², G. Kaya¹, E. Ergul², G. Kilic¹.* 1) Department of Neurosciences, Istanbul University, DETAE Institute., 34500, Istanbul, Turkey; 2) Department of Medical Biology and Genetics, Faculty of Medicine, University of Kocaeli, Derince, 41900, Kocaeli, Turkey.

The C677T and A1298C mutations in the methylenetetrahydrofolate reductase gene are associated with hyperhomocysteinemia, increased risk for venous thrombosis and atherosclerosis in homozygotes and in the compound heterozygotes. Increased homocysteine levels in serum can act as an excitatory amino acid and may affect the threshold of migraine headache. We studied the frequency of C677T and A1298C mutations and their association with migraine in 102 Turkish patients with migraine and 120 controls. Hundred and two patients with migraine headaches (23 with aura, 70 without aura), 2 with complicated migraine, and 7 with basal migraine, and 120 normal controls participated in the study. The mean age was 37.16 ± 9.66 in cases and 40.68 ± 13.39 in controls (90.7 % female and 9.3 % male). Genotyping of MTHFR C677T and A1298C polymorphisms was performed by PCR-RFLP method. Population frequencies of the MTHFR C677C, C677T and T677T genotypes were 40.2 %, 52% and 7.8 % in the cases and 50.8%, 44.2% and 5 % in the controls, respectively. The allelic frequency of C677T was 33.82 % in the cases and 27.08% in the controls. And also population frequencies of MTHFR A1298A, A1298C and C1298C genotypes were 44.1%, 44.1%, and 11.8% in the cases and 45%, 46.7% and 8.3% in the controls, respectively. The allelic frequency of MTHFR A1298C was 33.82 % in the cases and 31.67% in the controls. The MTHFR C677T/C1298C was higher among 102 patients compared with 120 controls, conferring a 7.88-fold increase in risk for migraine (OR=4.857; 95% CI=0.534-44.168). We also observed a 4.9 and 2-fold increases in risk for migraine in individuals with the MTHFR T677T/A1298A and C677T/A1298C variant alleles, respectively (OR=3.041; 95 % CI=0.577- 16.022, and OR=1.228; 95 % CI= 0.641- 2.352). In conclusion, our data clearly suggest that The C677T and A1298C genotypes of the MTHFR gene is a genetic risk factor for migraine.

Candidate gene analysis in neural tube defects. *R. Klootwijk¹, M. Schijvenaars¹, P. Groenen², F. Hol¹, J. Willemen¹, R. Steegers-Theunissen^{2,3}, E.C.M. Mariman⁴, B. Franke¹.* 1) Dept of Human Genetics, University Medical Centre Nijmegen, Nijmegen, The Netherlands; 2) Dept of Epidemiology & Biostatistics, University Medical Centre Nijmegen, Nijmegen, The Netherlands; 3) Dept of Gynaecology & Obstetrics, University Medical Centre Nijmegen, Nijmegen, The Netherlands; 4) Dept of Human Biology, Faculty of Health Sciences, Maastricht University, The Netherlands.

Neural tube defects (NTD) are congenital malformations which arise through incorrect formation of the neural tube. NTD belong to the group of multifactorial diseases, caused by a combination of unfavorable environmental conditions and genetic factors. In humans up to 70 % of NTD can be prevented by maternal periconceptional folic acid supplementation. In addition, the nutritional factor myo-inositol and the trace element zinc play a role in the etiology of human NTD. At the moment not much is known about the molecular basis of the effects of inositol and zinc on neural tube formation. One approach to study the interaction between inositol or zinc and NTD in man is mutation-analysis of selected inositol- and zinc-related candidate genes in large NTD patient panels. Using this approach we screened the candidates AP-2 and ZIC2 by SSCP analysis. Screening of ZIC2 resulted in the detection of a novel frequent C1062T change (H354H) and the deletion of an alanine residue (A8 variant) in the proximal aminoterminal alanine stretch of ZIC2. The A9 variant is conserved between man and mouse. The A8 variant was not found in a selected set of controls, indicating a possible involvement of this variant in the etiology of human NTD. SSCP analysis of AP-2 resulted in the identification of a novel frequent C16238T change (N5479N). Transmission Disequilibrium Testing (TDT) was performed for the genes AP-2 and ZIC2 using the novel polymorphisms, which resulted in a borderline significant association between human NTD and AP-2 ($\chi^2=2.92$, $df=1$, $p=0.09$), and no significant association between human NTD and ZIC2 ($\chi^2=0$, $df=1$, $p=1$). The TDT results indicate a possible involvement of AP-2 in human NTD.

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Tau as a susceptibility gene for Parkinsons disease. *A.A. Pasqua, F. Annesi, P. Spadafora, D. Civitelli, P. Tarantino, I.C. Cirò Candiano, S. Carrideo, P. Serra, E.V. De Marco, G. Annesi.* Inst. of Neurological Sciences, National Research Council, Mangone, CS, Cosenza, Italy.

Parkinsons disease (PD) is primarily an alpha-synucleinopathy, rather than a taupathy, but there is evidence for an indirect association of tau gene with the pathogenic process in PD. The aim of our study was to verify if the GT dinucleotide repeat marker of the tau gene is associated with PD. We designed a case-control study using sporadic and familial PD subjects and not matched healthy control subjects all coming from the same geographical area. We determined the GT repeat polymorphism of the tau gene in 300 PD patients (180 men and 120 women) and 197 controls (92 men and 105 women). The median age at the time of the study was 66.32 years for PD patients and 74.89 years for controls. Both populations were in Hardy-Weinberg equilibrium. Allelic and genotypic distributions of GT repeat were compared between cases and controls, the relative risk was estimated through estimation of odds ratio (OR) with 95% confidence intervals (CI). The allele and genotype distributions of the GT-STR polymorphism of the Tau gene were significantly different between cases and controls. The allele A3 was more frequent in cases than in controls (30% vs.16%, $p < 0.001$). Individuals carrying the A3 allele had an increased risk of developing PD (OR, 2.6; CI, 1.75-3.70). We found an association of the A3 allele of the Tau gene with PD. Others studies reported a significant association of A0 allele and A0/A0 genotype with PD. The discrepancy among results in different studies suggests that the association between a given disease and genomic characteristics must be confirmed by different investigations in different populations.

Performance of different models predicting BRCA-mutation carrier status in 570 Italian families. *F. Marroni¹, P. Aretini², J.E. Bailey-Wilson³, G. Parmigiani⁴, G. Bevilacqua², S. Presciuttini^{1,3} and Italian Consortium for Hereditary Breast/Ovarian Cancer.* 1) Dept. Biomedicine, Univ. of Pisa, Italy; 2) Dept. Oncology, Univ. of Pisa, Italy; 3) NHGRI-NIH, Baltimore, MD, USA; 4) Dept. Biostatistics, JHU, Baltimore, MD, USA.

The Italian Consortium for Hereditary Breast and Ovarian Cancer has recently established a database of pedigrees analyzed for both BRCA1 and BRCA2; it currently includes 570 families (134 with identified pathogenic mutation). Here, we show a comparison of the probabilities predicted by the program BRCAPRO and by a similar genetic model implemented in the program MLINK of the FASTLINK package. Contrasting results of the two programs should help identifying problematic situations, and may provide estimates of the robustness of the calculated values. In our MLINK model, frequencies of the mutated BRCA1 and BRCA2 alleles were 0.0006, and 0.00022, respectively. Age at cancer onset or at last observation was stratified in 7 groups, and 6 possible phenotypes were considered, for a total of 42 liability classes. Cancer incidences within each class were the means of the corresponding incidences used by BRCAPRO. Probabilities of being carriers of BRCA1 or BRCA2 mutations were computed separately in each family by both programs, and the obtained correlation coefficients were 0.917 and 0.878, respectively. The most discrepant families were those including subjects with multiple tumors (the two programs treat these subjects differently). Data were analyzed both in terms of observed/expected number of identified mutations in different family strata, and in terms of total log-likelihood. Sensitivity of the molecular tests was set to 0.7. For BRCA1, the number of identified mutations was close to expectation, though there was compensation between risk over-estimation in HBC families and risk under-estimation in HBOC families; in BRCA2, the total number of identified mutations was remarkably higher than expectations, and this effect was mostly due to HBC families. We intend to estimate genetic parameters of the BRCA genes that would be appropriate for the Italian population by maximizing the likelihood of the genetic test outcome.

Platelet glycoprotein IIb/IIIa PI^{A2}/PI^{A2} homozygosity associated with 5-fold risk of myocardial infarction in young men - The Copenhagen City Heart Study. *S.E. Bojesen¹, K. Juul¹, P. Schnohr², A. Tybjaerg-Hansen^{2,3}, B.G. Nordestgaard^{1,2}.* 1) Clinical Biochemistry, Herlev University Hospital, Herlev, Denmark; 2) Copenhagen City Heart Study, Copenhagen, Bispebjerg University Hospital; 3) Clinical Biochemistry, Rigshospitalet, Copenhagen University Hospital.

Purpose: To explore association between the platelet glycoprotein IIb/IIIa PI^{A1}/PI^{A2} polymorphism and ischemic cardiovascular disease in a large population-based prospective study. **Methods:** Using the Copenhagen City Heart Study (n=9242) with 22 years follow-up, we assessed risk of ischemic cardiovascular disease and myocardial infarction in PI^{A1}/PI^{A2} heterozygotes or PI^{A2}/PI^{A2} homozygotes versus PI^{A1}/PI^{A1} non-carriers, stratified for gender and age.

Results: In this cohort from the Danish general population, 70.0%, 27.3% and 2.7% were non-carrier, heterozygous and homozygous, respectively. Incidence of ischemic cardiovascular disease was 172 and 109 per 10.000 person-years in homozygous and non-carrier men (log rank: $p=0.006$), while this difference was not observed in women ($p=0.33$). Age-adjusted relative risks of ischemic cardiovascular disease in homozygotes versus non-carriers was 1.5 (95% CI: 1.1-2.2) and 0.7 (0.4-1.4) in men and women (genotype-gender interaction: $p=0.03$). In men aged <40 years, 40-50 years and >50 years at entry, age-adjusted relative risks of ischemic cardiovascular disease were 3.5 (1.4-9.0), 2.4 (1.3-4.6) and 1.0 (0.6-1.8), respectively (genotype-age interaction in men: $p=0.03$). Equivalent age-adjusted relative risks of myocardial infarction in men were 5.3 (1.5-18), 3.5 (1.6-7.6) and 0.5 (0.2-1.5), respectively (genotype-age interaction in men: $p=0.002$). Finally, age at first myocardial infarction was 60 ± 2.5 and 65 ± 0.5 years in homozygous and non-carrier men (Mann-Whitney U-test: $p=0.04$). Homozygous and heterozygous women and heterozygous men did not differ from non-carriers in any of the above-mentioned analyses.

Summary: In men <40 years, 22-year risk of ischemic cardiovascular disease and myocardial infarction in platelet glycoprotein IIb/IIIa PI^{A2}/PI^{A2} homozygotes was 3- and 5-fold that of non-carriers.

Genetic variation in the oxidized LDL-receptor-1 (OLR1) gene and the risk of coronary artery disease. *Q. Chen¹, S.E. Reis¹, C. Kammerer¹, E. Luedeking-Zimmer¹, D.M. McNamara¹, D.F. Pauly², B. Sharaf³, R. Holubkov⁴, C.N. Bairey Merz⁵, G. Sopko⁶, M.I. Kamboh¹ and the WISE study.* 1) Univ Pittsburgh, Pittsburgh, PA; 2) Univ Florida, Gainesville, FL; 3) Brown Univ, Providence, RI; 4) Univ Utah, Salt Lake City, UT; 5) Cedars-Sinai Medical Center, Los Angeles, CA; 6) NHLBI, NIH, Bethesda, MD.

Several lines of evidence suggest that oxidized LDL (OxLDL) is a critical factor in the initiation of the atherogenesis process. OxLDL binds to scavenger receptors and subsequently internalized by macrophages, resulting in foam cell formation. A number of scavenger receptors have been identified, including the lectin-like OxLDL receptor-1 (LOX1 or OLR1). In an effort to understand the role of OLR1 genetic variation with coronary artery disease (CAD), we examined the distribution of three novel OLR1 polymorphisms in the Women's Ischemia Syndrome Evaluation (WISE) study population. The WISE cohort was divided into three groups: minimal/normal disease (<20% stenosis), moderate disease (20-49% stenosis) and significant disease (≥50% stenosis). The three OLR1 polymorphisms (intron 4/G@A, intron 5/T@G and 3'UTR/T@C) were in linkage disequilibrium ($p < 0.0001$). All three polymorphisms showed significant associations with CAD. The frequency of the 3' UTR/T allele carriers was higher in the significant stenosis group than the minimal/normal disease group (OR=1.81, $p < 0.0082$). When the data were stratified by APOE*4 and non-APOE*4 carrier status, the significant association was found to be confined among non-APOE*4 carriers (OR=2.02, $p < 0.0084$), suggesting an interaction between the OLR1 and APOE genes. To examine the functional significance of the 3'UTR sequence variation, we performed electrophoretic mobility shift assay (EMSA) using nuclear extract from aorta smooth muscle cells. Our data show that the sequence encompassing the 3'UTR polymorphism is involved in binding with a transcription factor and that there is an allele-specific binding of the 3'UTR sequence variation with a putative regulatory element. In summary, our data suggest that common genetic variation in the OLR1 gene may be associated with the risk of CAD.

Hepatic lipase mutations, elevated HDL cholesterol, and increased risk of ischemic heart disease; The Copenhagen City Heart Study. *R.V. Andersen¹, H.H. Wittrup¹, A. Tybjaerg-Hansen^{2,4}, R. Steffensen³, P. Schnohr⁴, B.G. Nordestgaard^{1,4}.* 1) Dept. Clinical Biochemistry, Copenhagen University Hospital, Herlev, Denmark; 2) Dept. Clinical Biochemistry, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; 3) Dept. Medicine B, Division of Cardiology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; 4) The Copenhagen City Heart Study, Copenhagen University Hospital, Bispebjerg, Copenhagen, Denmark.

Objectives: Hepatic lipase influences metabolism of high density lipoproteins (HDL). We investigated associations between single nucleotide polymorphisms (SNPs) in the hepatic lipase promoter, levels of HDL, and risk of ischemic heart disease (IHD). **Methods:** We genotyped 9121 20-93 year old whites from The Copenhagen City Heart Study, of whom 957 had IHD, and 921 Danish patients with IHD for the -216, -480 and -729 SNPs of the hepatic lipase promoter. **Results:** Frequencies of wild type, triple heterozygotes and triple mutation homozygotes in the general population were 61%, 33% and 5%, respectively. Compared with wild type, HDL cholesterol levels were 4% (0.06 mmol/L) and 10% (0.15 mmol/L) higher in heterozygotes and mutation homozygotes; the equivalent values for apolipoprotein AI were 3% and 7%. In prospective and case-control studies, mutation homozygotes versus wild type had relative risk and odds ratio for IHD of 1.5 (95% CI: 1.0-2.2) and 1.4 (1.1-1.9) when adjusted for age and 1.7 (1.2-2.4) when adjusted for age, sex and HDL cholesterol. In individuals with the apolipoprotein E 43 genotype, relative risk and odds ratio for IHD in mutation homozygotes versus wild type was 2.9 (1.5-5.6) and 2.0 (1.2-3.2) when adjusted for age, sex and HDL cholesterol. **Conclusions:** Hepatic lipase promoter SNPs are associated with increased HDL cholesterol and paradoxically an increased risk of IHD after adjustment for HDL cholesterol, and particularly in individuals with apolipoprotein E 43 genotype. Thus, in some instances, HDL elevations may mark atherogenic rather than anti-atherogenic states.

Association XBAI (APO B) genetic polymorphism in arterial coronary disease patients in mexican population. *L.*

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INTRODUCTION: The arterial coronary disease (ACD) is one of the principal causes of death in the world. Between the risk factors most important is find abnormalities in the lipids metabolism whose provide fort correlation with arterial coronary disease. Several studies have described the XbaI polymorphism association between gene Apo B and ACD, even so this association is contradictory in others parts of the world. **OBJECTIVE:** Determine the genotypic and allelic frequency of XbaI polymorphism in the gene Apo B in case-control study of incident Mexican with arterial coronary disease. **MATERIAL AND METHODS:** In this study, genomic DNAs from 89 arterial coronary disease patients and 60 controls in Mexican population were scanned. Analysis of XbaI polymorphism was done by amplification of to segment that included exon 26 (709 bp) of Apo B gene, which thas a site of recognition for the XbaI enzyme. Genotypes were identified like X1 (709 bp), X2(433 y 276 bp), by polyacrylamide gels to 6% staining with silver nitrate. **RESULTS:** The presence of the X1 genotype was identified in 55% of the cases and 50% of the controls whereas 38% of the cases and 45% of the controls were heterozygotes. A statistical difference was not found to compared the previous data (X2=1.1618, p=0.5725). **CONCLUSIONS:** Our data suggest that tendency exists of allele X2 in arterial coronary disease patients in our population.

Prevalence of factor V Leiden G1691A, prothrombin G20210A and methylenetetrahydrofolatreductase C677T mutation in patients with chronic venous insufficiency. *J. Fuchs, M. Podda.* Dept Dermatology, Univ Frankfurt, Aschaffenburg, Germany.

Chronic venous insufficiency (CVI) is a very variable and complex entity including a spectrum of clinical presentations ranging from varicose veins through chronic lower extremity pain and edema to various skin changes and ulceration. CVI affects a large number of people in Western countries, and is responsible for considerable inconvenience, discomfort, suffering, and costs. The pathophysiology of CVI is complex, and involves abnormalities in coagulation, fibrinolysis and proinflammatory cytokines. It is believed that the majority of CVI cases result from primary venous insufficiency involving an alteration in the elasticity of venous walls and the dysfunction of venous valves. Less frequent causes of CVI are primarily acute deep venous thrombosis (DVT), which can occur asymptotically, particularly in the distal part of the lower extremity, and may thus remain undiagnosed. It was recently reported that one of every three patients with deep venous thrombosis of the lower extremities will develop, within 5 years, CVI sequelae that vary from minor signs to severe manifestations. According to our knowledge there are no reports in the literature investigating the thrombophilia status of CVI patients in comparison to their functional venous status. The purpose of this ongoing study is to study the prevalence of factor V Leiden G1691A, prothrombin G20210A and methylenetetrahydrofolatreductase C677T mutation in CVI patients in comparison to a control group and to evaluate the venous function of these patients by color coded duplex scanning and plethysmography. For mutation detection a standardized real-time PCR protocol was used. The preliminary data indicate that factor V Leiden G1691A mutation is more frequent in CVI patients than in the control group and the general population. Thus patients with factor V Leiden mutation have an increased risk of developing CVI. However, the majority of our CVI patients had primary venous valve dysfunction leading to CVI and were not positive for factor V Leiden G1691A, prothrombin G20210A or methylenetetrahydrofolatreductase C677T mutation.

Association of ApoAV haplotypes and triglyceride levels in French Canadians. *J.C. Engert^{1,2}, J. Faith¹, Y. Renaud¹, C. Dore¹, P. Lepage¹, T.J. Hudson^{1,2}, D. Gaudet^{3,4}, M.-C. Vohl^{5,6}*. 1) Montreal Genome Ctr, L3-317, Montreal General Hosp, Montreal, PQ, Canada Montreal Genome Centre, McGill University Health Centre Research Institute, Montreal, Quebec, Canada; 2) Departments of Human Genetics and Medicine, McGill University, Montreal, Canada; 3) Dyslipidemia, Diabetes and Atherosclerosis Group and Community Genetics Research Center, Complexe Hospitalier de la Sagamie, Chicoutimi, Quebec, Canada; 4) Community Genomic Medicine Center and Department of Medicine, University of Montreal, Canada; 5) Lipid Research Center, CHUQ Pavillion CHUL, Quebec, Canada; 6) Department of Food Sciences and Nutrition, Laval University, Quebec, Canada.

ApoAV was recently identified as a member of the apolipoprotein cluster on chromosome 11q23 through comparison with the homologous mouse sequence. A search for sequence variants at the ApoAV locus in a heart disease sample population from the Saguenay-Lac-St-Jean (SLSJ) region of Quebec led to the identification of eight SNPs: two 5' flanking region variants, one in the 5' UTR, one intronic, three in the 3' UTR and one missense mutation. Four of these SNPs are on a previously described haplotype and four haplotypes account for over 97% of all chromosomes observed to date. One haplotype in particular, defined by a single SNP in the 3' UTR, led to significantly lower total plasma tryglyceride levels (TG) in both patients and controls. The affect appears to be specific to TG levels, as no other measured plasma lipid parameters were affected. More than 20 different variants have already been described in the apolipoprotein cluster on chromosome 11q23. Interestingly, the LD is high ($D' > 0.82$) between the previously described haplotype and the SstI variant in the 3' UTR of the ApoCIII gene located just over 40Kb away. This variant has been associated with increased TG levels in several populations, but only inconsistently. In addition, none of the ApoAV allelic variants or haplotypes had any significant association with heart disease frequency in this population. These data will focus future studies and guide analysis of haplotype transmission to characterize the involvement of this apolipoprotein cluster in TG levels and metabolism.

DNA yield as a possible confounder in genetic studies on cardiovascular diseases. *M. Alanne*¹, *V. Salomaa*², *J. Stengård*², *L. Peltonen*^{1, 3}, *M. Perola*^{1, 3}. 1) Molecular Medicine, National Public Health Institute, Helsinki, Finland; 2) Epidemiology and Health Promotion, National Public Health Institute, Helsinki, Finland; 3) Human Genetics, UCLA School of Medicine, Los Angeles, CA.

Most large studies nowadays collect samples for DNA analyses. When the quantity of DNA needed is important, blood leukocytes are preferred because the yield from whole blood is easily much greater than the yield from standard buccal cell procedures. In the FINRISK 97 population-based survey EDTA-blood was collected for the extraction of DNA. The total sample consisted of 7982 participants with DNA. We found, that though treated similarly, the yield from whole blood varies significantly between individuals reflecting most likely different leukocyte levels in the blood. We wanted to see whether this variation in DNA yields would associate with any intermediate or end point cardiovascular phenotypes and thus potentially bias the sampling. DNA yield for females was significantly larger than for males ($p < 0.0001$). The mean yield of extracted DNA was smaller in the cases with myocardial infarction than in healthy controls (211 vs. 236 mg, $p = 0.007$). Age had a small negative trend for the yield of DNA ($p < 0.0001$). From the inflammatory measurements, both fibrinogen and CRP predicted the DNA yield statistically significantly ($p < 0.0001$) in a linear regression analysis. The most important measured predictors of DNA yield were the white blood cell count ($r^2 = 0.05$, $p < 0.0001$), and the time from blood drawing to extraction ($r^2 = 0.08$, $p < 0.0001$). In a linear regression model, myocardial infarction predicted DNA yield at the $p = 0.057$ level of statistical significance, but only if not sex or age were included in the model. The results suggest, that if a study selects its participants by their DNA yield (purposefully or forced to it by the low amounts of DNA), this can bias the study setting from the very beginning. When the role of inflammation in the pathogenesis of many diseases is evidently significant, we suggest that DNA amount should not become a selection factor for any kind of genetic study.

Genetic and environmental correlations between stature growth curve parameters and age at BMI rebound. *S.A. Czerwinski, B. Towne, E.W. Demerath, W.C. Chumlea, S.S. Guo, R.M. Siervogel.* Lifespan Health Research Center, Community Health, Wright State University School of Medicine, Dayton, OH.

Epidemiological studies have shown that children who mature earlier have an increased risk of being overweight in adulthood. In addition, several studies have also shown that children who experience the BMI rebound earlier in life are more likely to be overweight in adulthood. Acknowledging that measures of adiposity and patterns of human growth are under moderate to strong genetic control, we examined the nature of these association using a bivariate variance components approach to estimate the genetic and environmental correlations between stature growth curve parameters during adolescence and age at BMI rebound.

Serial weight and stature data from 428 participants in the Fels Longitudinal Study were used to model two sets of growth curves. Stature data for each individual were fit using the triple logistic model. From this, three adolescent growth curve parameters were derived: peak height velocity (PHV), age at PHV, and height at PHV. Weight and stature were used to calculate BMI (kg/m^2). Serial BMI data for each individual were fit using a third degree polynomial function. From this, age at BMI rebound was derived. Heritability estimates (h^2) for the adolescent growth parameters are $h^2=0.57$ for PHV, $h^2=0.79$ for age at PHV, and $h^2=0.92$ for height at PHV. The heritability estimate for age at BMI rebound is $h^2=0.71$. Although all of the traits examined are highly heritable, preliminary analyses reveal little effect of shared genes between the adolescent growth curve parameters and age at BMI rebound; the genetic correlations are low and insignificant. There are, however, statistically significant environmental correlations between age at PHV and age at BMI rebound ($r_E=0.61$) and between height at PHV and age at BMI rebound ($r_E=-0.81$). These results suggest that shared environmental factors may be more influential than shared genetic factors in any demonstrated associations between the age at BMI rebound and aspects of the adolescent growth spurt.

Polymorphisms in the genes of renin-angiotensin system in Mexicans with Ischemic cardiomyopathy. *T.J. BELTRAN¹, R. ARECHAVALETA², M.P. GALLEGOS¹, J. ARIAS¹, R. CELIS³, J. SANCHEZ¹*. 1) Medicina Molecular, CIBO-IMSS, Guadalajara, Jalisco, Mexico; 2) Servicio de Endocrinología; 3) Departamento de Epidemiología, H.E., CMNO, IMSS.

Ischemic cardiomyopathy (IC) is a multifactorial disease which is evoked by multiple environmental and genetic factors. Recent progress in molecular biology enable us a comprehensive understanding of the molecular pathogenesis of this disease. The genes of renin-angiotensin system has been reported as a positive linkage to IC in other part of world. In Mexico unknown this association. In 93 controls and 115 patients with IC was evaluated by coronariography. The ACE insertion (I)/deletion (D), M235T angiotensinogen and A1166C angiotensin II receptor type 1 polymorphisms were analyzed. No significant association was found when were analyzed the M235T and A1166C polymorphisms in patients with IC. However, a significant association with IC was observed in the patients group (n=111) with ACE D allele ($p < 0.05$). The subjects with the ACE D allele genotype are associated with IC in Mexican population.

A Novel Missense Variation in the Catalytic Domain of Rho-kinase May Genetically Impact on Coronary Vasospastic Ischemic Heart Disease in the Japanese. *H. Kamiunten, G. Koike, H. Shimokawa, A. Takeshita.*
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Background—Coronary vasospasm is one of the major causes of ischemic heart disease (IHD) and its prevalence is higher in the Japanese than in the western population. We have recently shown that Rho-kinase (also known as ROCK2) plays a crucial role in the pathogenesis of coronary vasospasm in humans, however, the genetic impact of Rho-kinase gene variation on coronary vasospasm remains to be examined. *Methods and Results*—Among healthy Japanese subjects, we have identified a novel single nucleotide polymorphism (SNP), G930T, in the catalytic domain of Rho-kinase, which results in an amino acid substitution, Lys310Asn. Then, we genotyped our Japanese patients with IHD (n=125) and Caucasian patients with IHD from ENCORE I study (n=318) to elucidate whether it is associated with coronary vasospasm. We also examined additional 4 SNPs of interest, including angiotensin converting enzyme I/D polymorphism, angiotensinogen C704T, angiotensin II type 1 receptor A1166C, and endothelial nitric oxide synthase C-786T in each population. We tested the effects of intracoronary acetylcholine in 357 consecutive patients and classified them into 3 categories ; large epicardial coronary artery spasm group (LAS), microvascular spasm group (MVS), and normal group (N). The frequency of T930 allele had higher tendency in the LAS+MVS group (3/57 for the LAS group and 2/42 for the MVS group) than in the N group (0/26) in the Japanese ($p=0.0479$). Interestingly, no Caucasian patient had the T930 allele, suggesting this Rho-kinase SNP may explain in part the ethnic difference in the prevalence of coronary vasospasm. There was no remarkable association between coronary vasospasm and other 4 SNPs in both populations. *Conclusion*—The G930T SNP of Rho-kinase may have a genetic impact on IHD in the Japanese, resulting in a higher morbidity due to coronary vasospasm. Further studies with a larger population aiming to elucidate whether this novel SNP alters Rho-kinase function are ongoing.

Association of the Common ABCA1 Variant, R219K with Plasma Lipid Levels and Coronary Artery Disease in Korean Population. *Y. Ko, E. Cho, H. Park, Y. Jang, S. Kim, J.E. Lee.* Cardiovascular Genome Center, Cardiovascular Research Institute, Division of Cardiology, Yonsei University, Seoul, Korea.

Background : ABCA1 is known to play a key role in the reverse cholesterol transport. Recently, a coding single nucleotide polymorphism (SNP) of the ABCA1 gene, the R219K variant, was found commonly in European population and reported to be associated with increased HDL-C, decreased TG, and a reduced progression of atherosclerosis. The purpose of this study was to investigate the frequency of the R219K variant in Korean population and its association with plasma lipid levels and CAD. **Methods :** One hundred ninety six CAD patients (m:f = 148:48, 57.7± 8.3 years) and 935 healthy control subjects (m:f = 402:533, 43.9±11.8 years) were examined. The R219K polymorphism was investigated by using SNP-IT assay. The plasma lipid levels were determined and the findings of coronary angiography in CAD patients were reviewed. **Results :** A homozygous R219K variant genotype KK was found in 21.5% of normal control subjects. The carrier frequency of K allele was 72.0%. The distribution of ABCA1 genotypes in CAD patients did not differ significantly from that of normal control subjects. However, there was a significant association of this ABCA1 genotype variant with HDL-C plasma level, especially in male normal controls; mean HDL level was highest in KK (53.3±11.4) and lowest in RR genotype (49.2±11.7)(P=0.02). Also, in the male CAD patients, KK genotype showed a higher mean HDL level (39.7±9.1) than RR genotype (36.0±7.4), even though the difference was not significant. The female groups showed, however, no association between plasma HDL levels and the genotypes. The severity of CAD was also related to the ABCA1 genotype. Three-vessel diseases were found more commonly in RR genotype (KK vs RR: 15.6% vs 30.2%), whereas one-vessel-disease were present more frequently in KK genotype (KK vs RR: 60.0% vs 35.8%)(p=0.05). **Conclusion :** The R219K variant of ABCA1 gene was found more frequently in Korean population than in Europeans (46%) and was associated with increased HDL plasma levels and a reduced severity of CAD.

Left Ventricular Dilatation in a large Arrhythmogenic Right Ventricular Cardiomyopathy (OMIM# 60440) population: prevalence, incidence and diagnostic utility. *K. Hodgkinson¹, S. Stuckless¹, E. Dicks¹, S. Connors², L. Thierfelder³, J. Drenckhahn³, M. Norman⁴, W. McKenna⁴, P. Parfrey¹.* 1) Patient Research Centre, Health Science Centre, St John's, NF, Canada; 2) Division of Cardiology, Memorial University, St. John's, NF, Canada; 3) Max-Delbruck Centre, Berlin, Germany; 4) St. Georges Hospital Medical School, London, England.

Six large families with Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) comprise a population with a founder effect, linked to a 2cM haplotype at 3p23-25 in Newfoundland. The families comprise 118 sibships (n=386). 73 sibships (n=263) are well ascertained; i.e. they contain 50% or more individuals of known status as defined by high-risk (sudden death under 50 years, cardiac intervention for manifestations of ARVC, obligate carrier by pedigree or high-risk DNA haplotype) or low risk (low-risk DNA haplotype). Only individuals from well-ascertained sibships are included in the analysis of which, 134 (79 male, 55 female) are high-risk (HR), 70 (30 m, 40 f) are low-risk (LR) and 59 (36 m, 23 f) are unknown. 65 HR (49%) and 47 (67%) LR individuals had at least one echocardiogram (EC). Left ventricular dilatation (LVD) was defined as greater than 117% using the Henry formula (indexing left ventricular dimensions to height, weight and age). At first EC, mean ages for the HR and LR groups respectively were 35(SD 14.4) and 33(SD 11.6). Initial prevalence of LVD in the HR group was 23% (n=15). 34 individuals without LVD at baseline had more than one EC (15 m, 19 f) and a Kaplan Meier analysis showed that 50% developed LVD *de novo* by age 52yrs, with a trend to earlier age of onset for males. At last follow-up EC the mean ages of the HR and LR groups were 38(SD14.2) and 36(SD 10.9) respectively: specificity was 94% (90% m and 96% f) positive predictive value (ppv) was 90% (88% m, 91% f) and sensitivity was 38% (50% m, 28% f). In summary, LVD is common in this population with ARVC, it is age dependant with a trend to a sex-effect and it has a high ppv and specificity.

Heritability of premature coronary artery disease and its related traits based on a study of 428 ascertained multiplex families. *S. Rao*^{1,2}, *G. Shen*^{1,2}, *R. Cannata*¹, *E. Zirzow*¹, *R.C. Elston*³, *X. Li*⁴, *E.F. Plow*², *E.J. Topol*^{1,2}, *Q. Wang*^{1,2}. 1) Center for Cardiovascular Genetics, Department of Cardiovascular Medicine, Cleveland Clinic Foundation, Cleveland, Ohio 44195; 2) Department of Molecular Cardiology, Cleveland Clinic Foundation, Cleveland, Ohio 44195; 3) Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, Ohio 44109; 4) Department of Mathematics, Harbin Medical University, China 150086.

Coronary artery disease, the leading cause of death in the developed countries, is a complex disease with a mixed contribution of genetic and environmental factors. Quantitative genetic analysis can be used to distinguish the genetic and environmental components. We estimated genetic and environmental variance components of premature coronary artery disease (PCAD) and its related traits in 2030 participants from 428 multiplex families in the Cleveland Clinic Foundation Gene Quest Study. Mixed linear models were used to decompose their phenotypic variances into additive genetic, common environmental causes, after simultaneously adjusting the effects of measured systematic covariates. In order to confirm the results obtained via mixed linear models, family aggregation analysis was carried out to obtain the correlations between various types of relative pairs. The two types of analyses agreed each other to confirm the existence of genetic cases in the manifestation of PCAD and its related phenotypes. The estimated heritability, a ratio of additive genetic variance over the phenotypic variance, was 0.45 for PCAD, and varied considerably among its related phenotypes. We concluded that premature coronary disease has an important heritable component. However, full elucidation of the genes responsible for this common complex disease must be complemented by lifestyle preventive strategies to deal effectively with a large, non-heritable component.

Sequence variation in hypoxia-inducible factor 1 alpha (*HIF1A*). *S.M. Roth*^{1,2}, *S.J. Prior*², *D.A. Phares*², *M.D. Brown*², *J.M. Hagberg*², *R.E. Ferrell*¹. 1) Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA; 2) Dept Kinesiology, Univ Maryland, College Park, MD.

Hypoxia-inducible factor 1 (HIF-1) is an oxygen sensing transcription factor consisting of two subunits, alpha and beta, with the alpha (HIF-1alpha) subunit unique to HIF-1 and regulated by hypoxia. We pursued polymorphism screening of *HIF1A* (14q21-q24) as a candidate gene contributing to inter-individual variability in maximal oxygen consumption and the response of oxygen consumption to exercise training. To date, we have completed screening of 40% of the exonic, exon/intron boundary, and promoter regions of *HIF1A* and have identified five variants as follows: 2 promoter variants (-2500 and -2209), and three polymorphisms in introns 4, 6, and 9. The two promoter variants appear to influence transcription factor binding sites (CdxA and AhR/Arnt, respectively), with the A-2500T site being more common in our initial screening. We genotyped 136 Caucasians and 59 African Americans for the A-2500T promoter variant and determined that the rare T allele was present only in African Americans ($p = 0.66$, $q = 0.34$). Exercise-induced maximal oxygen consumption ($VO_2\text{max}$) data were available before and after exercise training for 14 African American subjects. Carriers of the T allele ($n = 6$) exhibited lower baseline $VO_2\text{max}$ compared to the A/A group (21.7 ± 0.9 vs 25.1 ± 0.8 ml/kg/min; $P = 0.02$) with no difference between groups for the change in $VO_2\text{max}$ with training (4.4 ± 1.5 vs 5.0 ± 1.2 ml/kg/min; $P = 0.74$). In addition to completing the screening of *HIF1A* for polymorphisms, additional African American subjects are being genotyped for the A-2500T *HIF1A* variant for analysis of oxygen consumption and other exercise training-related phenotypes.

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Association of the Angiotensinogen (AGT M235T) and Rh Blood Group Polymorphisms with Blood Pressure and Lipids in an African Derived Population in Dominica. *M.Thomas Robinson¹, C.E. Grim²*. 1) Psychiatry, Univ. of Calif. San Diego, La Jolla, CA; 2) Department of Cardiology, Medical College of Wisconsin, Milwaukee, WI.

We investigated the association of two Chromosome 1 polymorphisms (AGT M2235T and the Rh Blood Group) with blood pressure, lipids, and lipoprotein concentrations in a random sample of Afro-Caribbeans age 18-60. In monogenetic analysis, AGT M235T was not associated with blood pressure. However, it was significantly associated with HDL concentrations (MM 4223, MT 4412, TT 5214 ($p=0.002$)). Rh genotype was significantly associated with systolic blood pressure ($p=0.006$) and Apo-A levels ($p=0.003$). These effects were not due to AGT M235T effects on weight or BMI or due to age or gender differences between groups. In the polygenetic analysis AGT M235T and Rh achieved borderline significance in an association with systolic blood pressure ($p=0.0680$; interaction effects, $p=0.037$). The association of the AGT M235T with blood pressure across Rh blood group haplotypes was then tested. Of the five Rh haplotypes available for analysis, the AGT M235T was significantly associated with blood pressure within the D haplotype ($p=0.01$). The Rh blood group and sex were significantly associated with systolic blood pressure and Apo-A levels, ($p=0.005$ and $p=0.012$, respectively). All interactions were independent of age and weight. In conclusion, we demonstrated a significant association of the AGT M235T with blood pressure and cholesterol metabolism in an Afro-Caribbean population in the genetic context of the Rh blood group system. Further investigation of these interactions may help understand the effects of genetic factors on cardiovascular risk in African derived and other populations.

Diagnostic Utility of Premature Ventricular Complexes in a large Arrhythmogenic Right Ventricular Cardiomyopathy (OMIM# 60440) population. *S. Stuckless¹, K. Hodgkinson¹, E. Dicks¹, S. Connors², L. Thierfelder³, J. Drenckhahn³, M. Norman⁴, W. McKenna⁴, P. Parfrey¹.* 1) Patient Research Centre, Health Sciences Centre, St. John's, NF, Canada; 2) Division of Cardiology, Memorial University, St. John's, NF, Canada; 3) Max-Delbrück Centre, Berlin, Germany; 4) St. George's Hospital Medical School, London, England.

A founder effect in a large Newfoundland Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) population genetically links 6 large families to a 2cM haplotype at 3p23-25. Only individuals from well-ascertained sibships (comprising 50% or more individuals of known status n=263) were included in the analysis. Status was defined as high-risk (sudden death under 50 years, cardiac intervention for manifestations of ARVC, obligate carrier by pedigree or high risk DNA haplotype) or low risk (low-risk DNA haplotype). Of the well-ascertained individuals, 134 (79 male, 55 female) are high-risk (HR), 70 (30 m, 40 f) are low-risk (LR) and 59 (36 m, 23 f) are unknown. 61 (46%) of the HR group (29m, 32f) had at least one 24 hour holter monitor (HM) and 29 (41%) individuals at LR (12m, 17f). Numbers of premature ventricular complexes (PVCs) in a 24-hr period were counted. The mean number of PVCs in the high-risk group was 3324, with a median of 1340 compared with 30 and 1 in the low risk group respectively (p=0.001). To determine the diagnostic utility of HM, 5 PVCs per hour was taken as an upper limit of normal (120 PVCs in 24 hours). Using the last HM obtained specificity was 93% (91% m and 94% f) positive predictive value (ppv) was 96% (96% m and 95% f), and sensitivity was 72% (82% m and 62% f). Prevalence over time for males indicates that all HR males over 30 years were above the cut-off for PVCs. Prevalence in HR females above 30 years was 70% (90% by age 45), with a ppv of 100% over age 30 years. In summary, PVC's occur frequently at an early age in this genetically homogeneous, well-ascertained, geographically stable population, and they are associated with a high positive predictive value.

Increased telomerase activity in older individuals with Down syndrome and dementia. *E.C. Jenkins¹, M.T. Velinov¹, S.-Y. Li¹, L. Ye¹, W.B. Zigman², N. Schupf², S. Sklower Brooks³, W.P. Silverman².* 1) Department of Cytogenetics, New York State Institute for Basic Research in Developmental Disabilities (NYS IBR), Staten Island, NY; 2) Department of Psychology, NSY IBR, Staten Island, NY; 3) Department of Human Genetics, NYS IBR, Staten Island, NY.

Recently, we presented preliminary data indicating reduced mitotic index in short-term whole blood cultures from people with Down Syndrome (DS) and dementia (AJHG 67(4):Abs. 1314). Given that telomeres become shorter with every in vitro cell division, given that individuals with DS have increased vulnerability and earlier onset of dementia, given that individuals with DS have been shown to have shorter telomeres than age- and sex-matched controls with DS (Vaziri, 1993), and given that telomerase, a ribonucleoprotein enzyme, is necessary for the synthesis of highly conserved TTAGGG repeats, we hypothesized that telomerase activity may differ as a function of dementia status in pairs of age- and sex-matched individuals with DS. Dementia status in these individuals was determined based on documented history of progressive memory loss, disorientation, and functional decline over a period of at least one year. In addition, all subjects had received a diagnosis of probable dementia by their personal physicians. Eight pairs of subjects with cytogenetically confirmed trisomy 21, ranging in age from 47 to 64 years, were studied. Using a PCR-based test via the TRAPeze kit (Serological Corp., Norcross, GA), we have found increased telomerase activity in short-term PHA-stimulated lymphocyte cultures from the subject with dementia in six of eight subject pairs (i.e., 75%, $p=.054$ using a one-tailed Wilcoxon signed rank test). We hypothesize that individuals with DS and dementia will exhibit both increased telomerase activity and decreased telomere length on further study. (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 National Institutes of Health grants PO1 HD3587-15, HD37425-04, and RO1 AG014676-04.).

Variable expressivity of a COL1A2 gly-610-cys mutation in a large Amish pedigree. *D.J. McBride, E.A. Streeten, B.D. Mitchell, A.R. Shuldiner.* Medicine, University of Maryland, Baltimore, MD.

Mutations in the COL1A1 and COL1A2 genes have been associated with osteogenesis imperfecta (OI), a disease characterized by bone fragility and fracture. Phenotypic variation is well recognized among individuals carrying different COL1A1 or COL1A2 mutations. Variation among related subjects carrying the same mutation is also known, but the total number of affected family members is limited. We have identified a COL1A2 mutation in 48 members of a large pedigree of Old Order Amish ancestry. All affected individuals (age 1 month-86 years) can be traced to a single founding couple born ~150 years ago. We assessed fracture history and measured bone mineral density (BMD) at the spine and hip in a subset of 37 affected (age 6-86 years) and 33 unaffected individuals (age 6-80 years) from 20 nuclear families. The average total number of self-reported fractures in individuals with the variant allele is 1.9 (range 0-9) compared to 0.52 (range 0-4) in unaffected relatives. Mean L1-L4 BMD adjusted for standing height in study subjects >18 years of age was significantly reduced in affected family members (5.8 0.5 mg/cm) compared to family controls (4.7 0.6 mg/cm). Variation in height adjusted BMD variation was also observed within nuclear families. Clinical variation in disease severity was observed. For example, one subject presented at age 31 with severe basilar impression. Other clinical features associated with OI such as dentinogenesis imperfecta, stature, scleral hue, and bone deformity have variable presentation both within and between nuclear families. The cause for this phenotypic variation is unclear at this time, but may result from the interaction of genotype with genetic background (modifier genes) and/or environmental factors. Discovery of modifier genes that influence OI phenotype may lead to novel treatment strategies that can impact substantially on mortality and quality of life in terms of fracture history. More importantly, these studies may yield new insights into the molecular and physiological basis of the common forms of osteoporosis, which, in turn, can lead to new prevention and treatment strategies for a broader group of patients.

Association of suicidality in bipolar patients with the T102C polymorphism of the 5-HT receptor 2A gene and coding variants R611H of the Wolfram Syndrome 1 gene. *S. Li¹, J.F. Goldberg⁵, T.A. Sivakumaran², J.H. Kocsis⁵, M.M. Lesperance², M. Burmeister^{1,3,4}.* 1) MHRI, University of Michigan, Ann Arbor, MI; 2) Departments of Otolaryngology Head and Neck Surgery, University of Michigan; 3) Department of Psychiatry, University of Michigan; 4) Department of Human Genetics, University of Michigan; 5) Department of Psychiatry, Cornell University Medical College.

Suicidality is one of the most serious symptoms of bipolar disorder. Several genetic variants have been implicated in suicidality before. Here, we ask whether two such genetic variants, the T102C polymorphism of the serotonin 2A receptor gene and a coding variant, R611H, in the Wolfram syndrome 1 gene, are associated with suicidality within a group of 68 bipolar patients. Suicidality was evaluated at intake and again at 1-year follow-up at Cornell University. Previous studies have shown associations of 5-HT_{2A}-T102C polymorphism with suicidality in patients with major depression. WFS1 was considered as a candidate gene because of an increased risk of psychiatric disorders and suicide has been reported for heterozygote carriers of Wolfram syndrome 1 (WFS1), a rare, autosomal recessive neurodegenerative disorder. Patients with the 102 T/T genotype had a lower mean number of suicide attempts (0.29) than T/C (1.10) and C/C (1.00) genotype patients, consistent with previous findings that the C allele is associated with suicide ideation. However, this finding did not reach statistical significance ($p=0.117$). The major finding of this study was that patients with a 611 R/R genotype of WFS1 had a significantly higher mean number of suicide attempts (2.0) than those with the R/H and H/H genotype patients (0.79, $p=0.038$). There were no significant differences in allele and genotype distributions found between non-suicide attempters and suicide attempters, and no deviation from Hardy Weinberg equilibrium. These preliminary findings suggest that the 5-HT(2A) receptor gene and Wolfram syndrome gene may play a role in suicidality risk of bipolar patients.

Increased frequency of the Apolipoprotein E e4 allele in patients with Down Syndrome. *J. Rodriguez, L. Rengifo, A.H. Alegria, M. Sanchez, P. Osorio, H. Serrano.* Basic Science , Universidad Tecnologica de Per, Pereira, Risaralda, Colombia.

Alzheimers disease (AD) is the most frequent entity in patients with Down syndrome (DS) who live more than 40 years. DS patients develop Alzheimers disease 20-25 years earlier than the normal population with a frequency 4-5 times higher. They all have neuropathology and neurochemical changes corresponding Alzheimers disease but not all develop the clinical symptomatology. It has been established that lipoprotein E e4 allele carriers have a dose dependent risk of developing Alzheimers disease at an earlier age while the e2 allele has a protective effect. On the other hand the e4 allele frequency in patients with Down syndrome has been found statistically similar to that of control populations although in young mothers with a meiosis II error, e4 frequency was 30.0%, significantly higher than in older mother with the same type of error (13% $p=0.03$) Apolipoprotein E genotyping was carried out by PCR amplification of a 244 bp fragment of the apoE gene followed by digestion with HhaI, in 78 patients (37 males, 41 females) with ages between 7 months and 41 years with a clinical and cytogenetic diagnosis of DS. A control group of 525 normal individuals (225 males, 300 females) with ages between 15 and 81 years was similarly analyzed. The e4 allele was found in 17% (95%, CI 15.0-18.3) of DS patients compared with 9% (95%, CI 8.6-9.9) in the normal population. This result is therefore statistically significant and differs from the results found by other mentioned previously. The mothers of the e4 allele carriers DS patients were 34-46 years old, indicating that the patients in the group studied here were conceived by older mothers.

A closer look at human recombination. *J. Barnard*¹, *D.F. Gudbjartsson*², *J. Sainz*², *G.M. Jonsdottir*², *S.A. Gudjonsson*², *B. Richardsson*², *S. Sigurdardottir*², *B. Hallbeck*², *G. Masson*², *A. Shlien*², *S. Palsson*², *M. Frigge*², *Th. Thorgeirsson*², *J. Gulcher*², *A. Kong*², *K. Stefansson*². 1) Statistics Department, DeCODE genetics, Waltham, MA; 2) Statistics Department, deCODE genetics, Reykjavik, Iceland.

Recombination, the mechanism of DNA mixing from one generation to the next, is a major component of the forces that drive human evolution. A recent study of ours (Kong et al 2002) demonstrates a substantially higher correlation between recombination rates and sequence content (e.g. GC content, CpG motifs, and polyA/polyT stretches) than reported before. Most interestingly, we found that not only do mothers have variable recombination rates ('mother effect'), even gametes of the same mother have systematic difference in recombination rates ('gamete effect'). There is a positive correlation of recombination counts across chromosomes suggesting there is some underlying factor that affects recombination rates across the genome. Here we will report results that quantify the substantial contributions of the mother effect and the maternal gamete effect to the total variation of recombination counts. We will also take a close look at whether the underlying factor has an uniform effect on the whole genome or whether the effect is more pronounced on certain chromosomes or certain parts of the chromosomes, and investigate the possibility that the effect is correlated with sequence content. Finally, we will briefly review the novel methodological and computational tools employed in our analysis to handle the issues of incomplete data and crossover interference.

Evidence That UGT A1 gene is a genetic modifiers in adults suffering from hemolytic anemia. *s. pissard*^{1,2}, *A. Jacquette*¹, *F. Roudot-Thoraval*⁴, *D. Bachir*³, *F. galacteros*³, *M. Goossens*^{1,2}. 1) service de biochimie et genetique, hopital Henri Mondor, creteil, France; 2) Inserm U468, creteil, France; 3) Unite de genetic du globule rouge, hopital henri mondor, creteil; 4) service de biostatistique, hopital Henri Mondor, Creteil, France.

Sickle cell disease (SCD : b6 glu>val) is one of the most frequent monogenic disorder and causes an accelerated destruction of red blood cells in peripheral circulation. The chronic hemolysis of SCD results in an hyperbilirubinemia and jaundice with secondary cholelithiasis potentially source of infections, abdominal pains and surgery. Serum bilirubine level is related to Uridine diphosphate Glucuronyl Transfrase 1A (UGT A1) enzyme activity and polymorphisms of the promoter of its gene are known to be strongly associated with UGT A1 activity in Gilbert syndrome. We have screened a cohort of 144 adults patients belonging to five geographic origins (French West Indies (46), western Africa (51), centre Africa (37) and Mediterranean area (7) and suffering either from SCD Hb S/Hb S (n = 115) or HB S/HB C (b6 E>K) (n = 23) and Hb S/b thal (n = 6) to evaluate the role of UGTA 1 as a genetic modifier of SCD and related hemolytic diseases. In these population we screened UGT A1 promoters' "TA" repeats and we found out 4 different alleles characterised respectively by 5, 6, 7 and 8 TA repeats the most frequents being the 6 and 7 repeats' alleles with no significant difference in the frequency of each allele between the populations (chi square = 4.2 df = 9, P = 0,89). Analysed on the 115 S/S patients, the frequency of cholelithiasis and related complications appear to be related to the number of UGT A1 promoters' repeats found in patient with 48.9% (genotype 6/6), 70,2% (genotype 6/7) and 79.3% (genotype 7/7) (p = 0.033). however, allele shorter than 6 repeats or longer than 7 repeats where not associated with decreased or increased severity. These data bring evidence for the role of UGT A1 gene as a genetic modifier of diseases characterized by chronic hemolysis and militate for the determination of UGT A1 genotype in the care of these patients.

Endometriosis in Large Utah Families. *K. Ward*^{1,2}, *D. Hull*². 1) Dept Obstetrics/Gynecology, Univ Utah, Salt Lake City, UT; 2) emerGen, Inc., 2749 E. Parley's Way, SLC, UT.

Our goals were to confirm the familial nature and heritability of endometriosis in Utah and to pursue disease gene identification using large families with common ancestors. Using an IRB approved protocol 784 women were identified as having surgically diagnosed endometriosis from 24 community-based gynecology offices. Three generation family histories were taken. 620 women had a sister of reproductive age with an average of 2.3 sisters. Of the 1404 sisters, 15.2% also had been surgically diagnosed and 22.2% had chronic symptoms consistent with the condition. 8% of the proband's mothers had a surgical diagnosis and 14.4% had suggestive symptoms. The patients were also questioned about their extended family. 35.5% reported at least one extended family member surgically diagnosed. The results are shown in the table below:

	Relatives	Probands	%	%	%
	affected	reporting	Affected	Male side	Female side
Cousin	153	110	14	10.2	3.8
Aunt	133	113	14.4	8.5	5.9
Niece	42	34	4.3	3.7	0.6
Grandmother	22	22	2.8	2.0	0.8
Daughter	40	39	5.0	-----	-----

This study confirms the familial nature of endometriosis and the likelihood of a major gene effect. Affected sibling pair linkage analysis of 210 affected sibling pairs is currently underway.

Trans-species polygenic variation of complement C4 and RCCX modular structures in rhesus macaque (*Macaca mulatta*) and humans. N. Longman-Jacobsen¹, E.K. Chung^{1,2}, Y. Yang^{1,2}, K.R. Clark¹, P. Johnson¹, C.Y. Yu^{1,2}. 1) Children's Research Institute, Columbus, OH; 2) Ohio State University, Columbus, OH.

The evolution of complement C4 in Old World primates appears to have been a complicated process, with humans having complex genetic variations of a four-gene segment containing RP-C4-CYP21-TNX (RCCX module) in the major histocompatibility complex (MHC). Besides humans, little is known regarding the genetic complexity of C4 and RCCX modular variation within each primate species. We have begun an examination of C4 genes and the RCCX complex in a large population of *Macaca mulatta* (Rhesus macaque), a desirable model for studies of C4 and other immune response proteins in infectious and autoimmune diseases. We show that several of the tools for the elucidation of the C4 gene copy number and RCCX modular structures in humans are also informative in macaques. We are able to determine the total number of RCCX modules and thus C4 genes by pulsed field gel electrophoresis of *PmeI* digested genomic DNA, and whether each C4 gene contains the endogenous retrovirus ERV-K(C4) by *TaqI* RFLP. The genotype results reveal that macaques have a similar level of complexity to humans, with mono-, bi- and trimodular RCCX structures that incorporate one, two or three C4 genes on each MHC, respectively. The monomodular short RCCX, which contains one C4 gene without an ERV-K(C4), is relatively common in macaques with a frequency ~31%. In humans, this haplotype has a frequency of 11% in the healthy Caucasian population. Sequencing of the genomic C4d region in macaques reveals a similar level of polymorphism to that seen in humans. One of the nucleotide changes leading to the Cys>Ser 1102 in human C4A and C4B isotypes, respectively, and the isotype definitive *PshAI* RFLP, appears to be invariant in macaques and is identical to human C4A. Sequencing will also allow production of recombinant proteins for the generation of specific antisera against macaque C4, as allotyping using human C4 antisera for immunofixation has not been informative. In summary, we have observed trans-species polymorphism with great genetic diversity in the RCCX modules and polygenic variations of C4 in rhesus macaques and in humans.

Natural Selection as a Curved Plane: Using Mathematic to Model Evolutionary Forces. *C.E. Grim¹, M.T. Robinson², D. Read³, T. Paz⁴, N.J. Schork².* 1) Cardiology, Medical College of Wisconsin, Milwaukee, WI; 2) Department of Psychiatry, University of California, San Diego. La Jolla, CA; 3) Department of Anthropology, University of California, Los Angeles. Los Angeles, CA; 4) Department of Epidemiology, University of Southern California, Los Angeles, CA.

Until recently, many computer systems were capable only of producing graphical display of numeric data in two dimensions. Advances in hardware and software have made the graphical display of numeric data, and its manipulation in three dimensional space possible. In this report we demonstrate the use of Mathematica for modeling the evolutionary force of natural selection and its interface with mutation. Four types of natural selection were examined: no dominance, overdominance, complete dominance with selection against the A2A2 genotype, and complete dominance with selection against the A1 allele. The four equations representing the rate of allele frequency change as a function of selection coefficient and initial allele frequency, derived by D.S. Falconer in *Introduction to Quantitative Genetics*, were used in these models. The change of allele frequency was plotted on the Z axis as a function of gene frequency (X axis) and the selection coefficient (Y axis). In this three dimensional representation, natural selection (the change in gene frequency) is a curved plane. Examination of these three-dimensional plots reveals that the form of natural selection have the largest potential impact on the allele frequency is that of no dominance. Under no dominance, Δq reaches a maximum value of 0.48 when the selection coefficient is 1.0 and the gene frequency is 0.5. Visualization of evolutionary forces in three dimensions, as we have demonstrated, enforces the concept that evolution is dynamic process, and encourages an intuitive understanding of the combination of factors, and their magnitudes, that are needed to produce a desired genetic result in a population.

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Detection of Population Structure in Starr County Mexican-Americans. *B.F. Voight, N.J. Cox, J.K. Pritchard.*
Human Genetics, University of Chicago, Chicago, IL.

Population structure is potentially a critical problem in association and in linkage disequilibrium based fine-mapping. Subdivisions that are present and are not appropriately accounted for can give rise to spurious associations between marker and phenotype that are intrinsic to population structure but are not intrinsic to disease. Admixed populations provide a particular challenge for computational approaches to detect subdivision, since the existing substructure may have a continuous distribution, not amenable to classification into discrete units, yet still show differences in marker and/or disease allele frequencies between a patient and a random or control sample. We have examined a population of Mexican-Americans from Starr County, TX with type 2 diabetes as well as a random sample also collected from Starr County using computational approaches developed by Pritchard et al (2000), implemented in the program STRUCTURE, to estimate the number of subpopulations in the sample. We utilized markers from a whole genome screen of 491 microsatellite loci typed in 170 patients (one patient from each of 170 families included in the genome screen) affected with type 2 diabetes. The results show little evidence of population subdivision. In order to maximize our power to detect subdivision within this admixed sample, we are further investigating a subset of the polymorphisms for which allele frequency estimates are available for populations of European, Spanish, and Native American descent.

Association analysis of single nucleotide polymorphisms of serotonin transporter gene and bipolar affective disorder in Taiwan. *H.S. Sun¹, H.C. Wang¹, T.J. Lai²*. 1) Institute Molecular Medicine, National Cheng Kung University Medical College, Tainan, Taiwan; 2) Department of Psychiatry, Chung Shan Medical and Dental University Hospital, Taichung, Taiwan.

Bipolar affective disorder is a chronic, severe mood disorder that is considered as the major mental illness after depression. Little is known about the underlying causes of BPD, which has estimated a lifetime prevalence of 0.5% to 1% in various populations. Current studies using the candidate gene approach have suggested that serotonergic neurotransmission and metabolism were associated with several psychiatric and behavioral traits including BPD, depression, suicidality, alcoholism, and mental control. Genes involved in serotonin transmission and metabolic pathways thus are good candidates for studying the involvement in BPD pathogenesis. Serotonin transporter (SLC6A4) is a member of solute carrier family and responds for re-uptaking serotonin into presynaptic terminal thus fine-tuning brain serotonergic neurotransmission. Serotonin transporter has been the primary target for developing therapeutic medicine for the treatment of depression, panic disorder and other psychiatric disorder. To examine the role of the SLC6A4 gene in etiology of BPD, 10 markers including two variable number of tandem repeat (VNTR) and 8 single nucleotide polymorphisms (SNPs) within 60 kb interval surrounding the SLC6A4 gene were used to study the association with BPD in Taiwan. Two selected SNPs were found not informative in Taiwanese population and eight polymorphic markers have been analyzed by classical chi-square test as well as haplotype analysis. No association can be obtained between all SLC6A4 markers and BPD. In addition, significant linkage disequilibriums were obtained among 8 SNPs but not between markers and a putative disease locus by the use of EH program. This study demonstrates that the SLC6A4 gene may not play an important role in BPD etiology and suggests that other genes in the serotonergic system may be involved.

FREQUENCY OF NON-SYNDROMIC DEAFNESS IN BOGOTA, COLOMBIA: RESULTS OF A SCREENING PROGRAM. *M.L. Tamayo¹, M. Olarte¹, N. Gelvez¹, P. Gonzalez¹, S. Florez², D. Medina².* 1) Instituto de Genetica Humana, Facultad de Medicina, Universidad Javeriana, Bogota, Colombia; 2) Fundacion Oftalmologica Nacional.

Hearing loss is one of the most common human complaints, affecting one in 1000 children. In developed countries, half of the hearing loss is due to a genetic cause. In Bogota, Colombia, we are developing a screening program in order to define the prevalence of Non-syndromic deafness in our City. We applied a special questionnaire to each affected child in all of the institutions for the deaf, asking for possible causes of deafness, presence of some affected relative, parents consanguinity and, other disorders or defects in the propositus. After that, we made a previous selection of some affected families and invited them to participate in our research. All selected individuals were examined by our medical team to define the diagnosis of non-syndromic deafness. We performed a complete clinical exam and a detailed ophthalmologic evaluation with fundoscopy, focus to rule out rubeola prenatal infection and other acquired causes. We classified the families in two groups: hearing loss from possible genetic origin, and hearing loss from unknown cause. Of the total of 731 individuals analyzed, we define a diagnosis of non-syndromic deafness in 299 (40.9%). Among this, 146 (48%) were classified as non-syndromic deafness with possible genetic etiology, and 153 (50.3%) as non-syndromic deafness with unknown cause. Molecular genetic screening for the most common mutations in Cx26, 35delG and 167delT, must be performed to clarify the real basic etiology for the non-syndromic deafness in this population.

Transmission disequilibrium for alleles of MSX1 in a large South American population with cleft lip and palate.

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Oral clefts, including cleft palate (CP), cleft lip (CL) and cleft lip with palate (CLP) have been the focus of numerous studies by both epidemiologists and geneticists. Their frequent occurrence as well as their extensive psychological, surgical, speech and dental involvement emphasize the importance of understanding the underlying causes. Previous candidate gene analyses have suggested the involvement of MSX1, TGFA, and TGFB3 in Caucasian population. The purpose of this research was to test the hypothesis that TGFB3 and MSX1 are involved in the etiology of CLP or CP. This was tested by determining, through the use of nuclear family based approach, whether these genes were in linkage disequilibrium as measured by transmission distortion with either form of clefting. The availability of a large sample of complete parent-infant triads (324) on whom DNA is available allowed us to pursue a TDT analysis for genes previously associated with cleft lip and palate. The genes were selected based on previous results, including linkage, linkage disequilibrium, and animal model studies. Markers used to analyse 324 triads were for the MSX1 and TGFB3 genes. MSX1 had positive (p-value 0.05) biallelic TDT results for allele 4 in the overall dataset, and in triads with CP. TGFB3 had a borderline positive result (p-value = 0.06) biallelic TDT result for allele 1 in the CL subset only. For both genes independent confirmation was identified and provides support for the role of these genes in contributing to the genetic influence on cleft lip and palate in a Brazilian population. It may be through the sum of studies like this, we can provide a search for evidence of transmission distortion in candidate genes of high priority for the study of cleft lip and palate. The evidence that MSX1 and TGFB3 contribute to clefting can provide us with more complete understanding of the etiology of human nonsyndromic cleft lip and palate.

The phylogeography of Siberian Y-chromosome lineages. *M. Derenko*¹, *B. Malyarchuk*¹, *G. Denisova*¹, *I. Dambueva*², *Ch. Dorzhui*³, *F. Luzina*⁴, *O. Lotosh*⁴, *I. Zakharov*⁵. 1) Genetics Laboratory, Institute of Biological Problems of the North, Magadan, Russia; 2) Institute of General and Experimental Biology, Ulan-Ude, Russia; 3) Tuva State University, Kyzyl, Russia; 4) Institute of Professional Diseases and Hygiene Problems, Novokuznetsk, Russia; 5) Vavilov Institute of General Genetics, Moscow, Russia.

DNA variation of the non-recombining part of the Y-chromosome was examined in 434 samples representing ten aboriginal Siberian populations (Altai-Kizhi, Teleuts, Tofalars, Khakassians, East Tuvians (Todjins) and Shors from Altai and Sayan region, Buryats and Sojots from Baikal region, Koryaks and Evens from northeast Siberia region). Twelve haplogroups (HGs: 1, 3, 12, 16, 10, 26, 2, 2, 9, 4, 21, 18, according to nomenclature proposed by Jobling et al. (1998) with modifications) were observed by analyses of twelve biallelic polymorphic markers (92R7, SRY1532, Lly22g, RBF5, M9, 12f2, M89, RPS4Y, DYS287, SRY8299, M20, DYS199) and were unevenly distributed among the populations. The most frequent Y-chromosomes in Siberians belonged to HG1 (15.7%), HG3 (30%), HG16 (16.8%) and HG10 (23.3%). Haplogroups 1 and 3 showed the highest frequencies in the Altai and Sayan region populations (up to 80% in Shors). Haplogroup 10 showed regional variation, having the highest frequencies in the Baikal region populations (54.5% in Buryats) and in the northeast populations of Koryaks and Evens (50% and 60%, respectively). The highest frequencies of HG16 were observed in all regional groups of Siberians (25% in Tofalars, 22.1% in Buryats and 28.8% in Koryaks), whereas HG12, which is ancestral to HG16, showed maximum frequency in Tofalars (34.4%). Phylogenetic analysis revealed three monophyletic Siberian clusters, which consisted of northeast/Baikal region populations, Altai-Sayan region populations and Tofalars/ Sojots, respectively. The distribution pattern of Y-chromosome haplogroups as well as the results of phylogenetic analyses testify the considerable inter-population differentiation of Siberian groups studied ($F_{st}=0.157$). AMOVA results show that rather geography and linguistics than anthropology define the degree of genetic differentiation observed.

A high-density SNP allele frequency map reveals genomic signatures of natural selection. *J.M. Akey¹, G. Zhang¹, K. Zhang^{1,2}, L. Jin¹, M. Shriver³*. 1) Dept Environmental Health, Univ Cincinnati, Cincinnati, OH; 2) Human Genetics Center, University of Texas-Houston, Houston, TX; 3) Department of Anthropology, Penn State University, University Park, PA.

Identifying genomic regions that have been targets of natural selection remains one of the most important and challenging areas of research in genetics. To this end, we report the analysis of 26,530 SNPs whose allele frequencies were determined in three populations. Specifically, we calculated a measure of genetic differentiation, F_{ST} , for each locus and examined its distribution at the level of the genome, the chromosome, and individual genes. By comparing the empirical distribution of F_{ST} to that predicted under a model of selective neutrality we observe clear and statistically significant signatures of natural selection at each level of observation. Furthermore, we have identified 174 candidate genes whose distribution of genetic variation suggests that they have been targets of selection. Our work provides a first generation natural selection map of the human genome and provides compelling evidence that selection has shaped extant patterns of human genomic variation.

DNA sequence variation in FOXP2 language gene among humans and great apes. *J.J. Ely^{1,2}, H.H. Khun¹, S.T. Boysen³, J.M. Erwin^{1,2,4}, P.R. Hof^{2,5}, W.I. Frels¹*. 1) Comparative Neurogenomics Laboratory, BIOQUAL, Inc, Rockville, MD; 2) Foundation for Comparative and Conservation Biology; 3) Department of Psychology, Ohio State University; 4) Obesity & Diabetes Research Center, University of Maryland School of Medicine; 5) Kastor Neurobiology of Aging Laboratories, Mount Sinai School of Medicine.

A single G-to-A transition in the FOXP2 gene causes developmental verbal dyspraxia, a severe language disorder involving deficits in the ability to produce fine orofacial movements and in the ability to parse words into constituent phonemes necessary for speech production and comprehension. With appropriate training, great apes have demonstrated the capacity for representational symbol production and comprehension but such capabilities are not seen under natural conditions. We hypothesized that DNA sequence variation in FOXP2 could account for innate differences in language ability among humans and apes. We sequenced 3099bp of genomic DNA including 2066bp in exons and 1933bp in introns, from exons 4-17 in 4 chimpanzees, 2 bonobos, 2 gorillas and 2 orangutans. One noncoding and 2 synonymous transitions, 1 synonymous transversion and 3 intronic SNPs distinguish species but neither the disease-causing R553H mutation nor any other functional differences were observed in the Fox region proper (exons 12-14). However, a 3bp insertion added 1 extra Gly residue in exon 5, while 1 transition and 1 transversion resulted in 2 conservative amino acid changes (N to T and S to N) in exon 7 in chimpanzee and bonobo near the putative zinc finger domain in exon 8. Language is a complex trait involving many different cognitive and motor skills controlled by many independent genes. Although the human neural architecture and motor cortex representation underlying spoken language evolved from phylogenetically ancient brain structures, the cytoarchitecture was probably considerably modified by new cortical circuits subserving the uniquely human motor aspects of language. These changes certainly involved more genes than FOXP2 alone. Thus, the relatively minor genetic changes observed in FOXP2 gene are unlikely to explain species differences in language competence.

Microsatellite and SNP haplotype variability at the SLC11A1 locus in globally diverse human populations.

SLC11A1. A.A. Awomoyi¹, M. Newport², G. Sirugo³, K. McAdam³, S. Tishkoff¹. 1) Dept Biol, Univ Maryland, College Park, MD; 2) CIMR, Wellcome Trust/MRC Building, Addenbrookes's Hospital, Hills Road, Cambridge. CB2 2XY; 3) MRC Laboratories, Fajara, The Gambia.

SLC11A1 Solute carrier 11a1 (formerly called NRAMPI natural resistance associated macrophage protein 1) is a pH dependent divalent cation transporter involved in macrophage function. SLC11A1 is a candidate gene for susceptibility to mycobacterial infections. Studies in humans indicate that polymorphisms at the 5' and 3' end of the gene are associated with increased susceptibility to tuberculosis (TB). Two alleles of a 5' promoter region polymorphism have been shown to have opposing effects on gene expression. Allele 3 results in overexpression and has been functionally linked to autoimmune disease susceptibility, while the decreased level of expression of SLC11A1 caused by allele 2 contributes to infectious disease susceptibility, leading to the hypothesis that polymorphisms at SLC11A1 are maintained in human populations by balancing selection. Hence, alleles that are detrimental in relation to susceptibility to infectious disease may be beneficial for decreased risk of autoimmune disease. In order to reconstruct the evolutionary history of the SLC11A1 gene and to distinguish the role that mutation, migration, drift, and selection have had on shaping the pattern of genetic diversity at this locus in humans, we are studying microsatellite and SNP haplotype diversity at this locus. Eight variable microsatellite repeats, including the promoter polymorphism, spanning a 3 MB region encompassing the SLC11A1 locus as well as 5 SNPs within the SLC11A1 locus have been genotyped in a globally diverse set of human populations (n>450) from Africa (Tanzania, Nigeria, Gambia), the Middle East (Lebanon), China, N. Europe, South America (Peru). Estimation of haplotype frequencies shows that Africans have higher heterozygosity than non-Africans. Analysis of patterns of haplotype variability and linkage disequilibrium have been used to reconstruct the evolutionary history of this locus and provide a clue about which haplotypes play a role in susceptibility to mycobacterial infection and/or autoimmune disorders.

Phylogeographic differentiation of mitochondrial DNA and Y-chromosome in Russian populations. B.

Malyarchuk¹, M. Derenko¹, G. Denisova¹, T. Grzybowski², E. Rogaev³, D. Miscicka-Sliwka², R. Villems⁴. 1) Genetics Laboratory, Institute of Biological Problems of the North, Magadan, Russia; 2) The Ludwik Rydygier Medical University in Bydgoszcz, Poland; 3) National Research Center of Mental Health, Moscow, Russia; 4) Department of Evolution Biology, Tartu University and Estonian Biocentre, Tartu, Estonia.

To characterize the mitochondrial DNA (mtDNA) variation in Russians, sequences of the first hypervariable segment (HVS I) of the control region were obtained from 391 individuals representing six populations of Russians from European part of Russia (Krasnodar, Stavropol, Belgorod, Orel, Saratov, and Nizhnij Novgorod regions). The analysis of HVS I variation in combination with RFLP typing of the coding region haplogroup-diagnostic sites revealed that 98% of mtDNA haplotypes found in Russians fall into major West Eurasian haplogroups. Comparative analysis of the mtDNA variation in Russian populations from different regions of European Russia has shown that despite the high levels of diversity in populations, mtDNA sequences are highly homogeneous across populations. Nevertheless, the low level of differentiation between southern and central-eastern Russian populations ($F_{ST} = 0.4\%$, $p < 0.04$) was found, when the neighboring Finno-Ugric populations are taken into account. In contrast to mtDNA, Y-chromosome analysis performed in southern, central and eastern Russian populations has revealed a high level of between-group differentiation ($F_{ST} = 2.6\%$, $p = 0.03$). It was found that hg9 Y-chromosomes reach the highest frequency of 14% in southern Russian populations. In addition, hg21 (YAP+) chromosomes are also concentrated in the south (7%). The frequency of hg16 varies in Russian populations from 7% to more than 30%, but its pattern of geographic distribution cannot be strongly explained as clinal, suggesting a more complex demographic history. This work was supported by the Russian Fund for Basic Research (grant 00-06-80448) and by the Ludwik Rydygier Medical University in Bydgoszcz (grant BW66/02).

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The Pattern of Polymorphism on Human Chromosome 21. *H. Innan, B. Padhukasahasram, M. Nordborg.* Molecular and Computational Biology, University of Southern California, Los Angeles, CA.

Polymorphism data from 20 partially resequenced copies of human chromosome 21 more than 20,000 polymorphic sites are analyzed. The allele-frequency distribution shows no deviation from the simplest population genetic model with a constant population size (although we show that our analysis has no power to detect population growth). The average rate of recombination per site is estimated to be roughly one half of the rate of mutation per site, again in agreement with simple model predictions. However, sliding window analyses of the amount of polymorphism and the extent of linkage disequilibrium (LD) shows significant deviations from standard models. This could be due to the history of selection or demographic change, but it is impossible to draw strong conclusions without much better knowledge of variation in the relationship between genetic and physical distance along the chromosome.

D1S80 variability in different ethnic groups of Russia and Belarus. *D.A. Verbenko¹, T.V. Kekeeva¹, E.K. Khusnutdinova², T.V. Pogoda¹, V.A. Spitsyn³, A.I. Mikulich⁴, N.A. Bebyakova⁵, L.V. Bets⁶, V.P. Ivanov⁷, N.N. Abolmasov⁸, L.A. Tarskaya¹, M.V. Sorensen⁹, S.A. Limborska¹.* 1) Human Molecular Genetics Dept., Institute of Molecular Genetics, Moscow, Russia; 2) Institute of Biochemistry and Genetics, Ufa Scientific Centre of Russian Academy of Sciences, Ufa, Russia; 3) Research Centre of Medical Genetics, Russian Academy of Medical Sciences, Moscow, Russia; 4) Institute of Arts, Ethnography and Folklore, National Academy of Sciences of Belarus, Minsk, Belarus; 5) North State Medical University, Arkhangelsk, Russia; 6) Moscow State University, Moscow, Russia; 7) Kursk Medical University, Kursk, Russia; 8) Smolensk Medical University, Smolensk, Russia; 9) McGaw Medical Center, Northwestern University, Chicago IL, USA.

High individual discrimination power D1S80 (pMCT118) DNA marker was used to learn different ethnic groups from Russia and Belarus. Allele polymorphism for this locus has been explored in Russians (six regions), Belorussians (six regions), Mordvinians-Moksha, Bashkirs, Komi-Zyrjans, Komi-Permjaks, Udmurts, Tatars, Mari, Kalmyks and Yakuts. Eastern Slavonic populations (Russians and Byelorussians) and also Mordovians-Moksha are Caucasoids; Kalmyks and Yakuts are Mongoloids; other populations are considered as admixture populations with different levels of a Mongoloid component. Allele typing was performed using the PCR and subsequent electrophoresis followed by silver staining. Twenty-eight alleles of D1S80 locus were noted in populations studied. The level of observed heterozygosity was appeared to be high and varied from 0.73 to 0.95. Observed allele distributions in Caucasoid populations studied are found to statistically differ from those in Mongoloid populations. D1S80 allele frequencies distribution pattern in Caucasian populations in contrast with others studied were characterized with lowest frequencies of high copy number alleles (more than 31) and allele 16. An analysis of D1S80 variability was made with multidimensional scaling treatment of Nei's genetic distances matrix. The plot obtained let to analyse population relationships and reveal high capability of D1S80 to differ both neighbouring and distant ethnic groups.

Mitochondrial haplogroup structure of Tibeto-Burman populations. *Y. Qian*¹, *J. Chu*², *R. Chakraborty*¹, *L. Jin*¹, *B. Su*¹. 1) 1. Center for Genome Information, Department of Environmental Health, University of Cincinnati, USA; 2) 2. Institute of Medical Biology, the Chinese Academy of Medical Sciences, China.

Using 10 East Asian-specific mitochondrial RFLP polymorphic markers, a total of 570 samples were screened covering nine Tibeto-Burman speaking populations from southwestern China as well as six populations who speak Altaic, Daic, Astro-Asiatic and one Japanese population. Consistent with previous observations in East Asia, the majority of the mtDNAs clustered into two major haplogroups, M (defined by 10394 DdeI+/10397 AluI+) and N (defined 10394 DdeI-/10397 AluI-), indicating a common maternal origin of Tibeto-Burman populations with other East Asians. Furthermore, the presence of the haplotype defined by 10394 DdeI+/10397 AluI- in most of these East Asian populations indicates its antiquity and possibly one of the ancient haplotypes (affiliated to L3 haplogroup) originated from Africa. The principal component (PC) analysis based on mtDNA haplotype frequency distribution revealed a close relationship between Tibetan and Japanese confirming the kin connection of these two populations in the paternal lineage. The Burmese-Lolo populations, a subgroup of Tibeto-Burman, are widely spread in the PC map, implying an extensive female population admixture between Burmese-Lolo and Daic/Astro-Asiatic speaking populations contrasting the relatively monomorphic genetic background in the paternal lineage of Burmese-Lolo.

Population structure and distribution of 100 polymorphic *Alu* insertion polymorphisms. *W.S. Watkins*¹, *C.T. Ostler*¹, *A. Brassington*¹, *M.J. Bamshad*¹, *M.A. Batzer*², *M.L. Carroll*², *S.V. Nguyen*², *L.B. Jorde*¹. 1) Dept. of Human Genetics, University of Utah, Salt Lake City, UT 84112; 2) Dept. of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803.

We have analyzed 100 *Alu* insertion polymorphisms in 155 Africans, 77 Asians, 118 Europeans, and 365 East Indians. *Alu* gene diversity is highest in Africans (0.349) and lowest in Europeans (0.298). The *Alu* insertion frequency is lowest in Africans (0.463) and higher in Indians (0.544), Asians (0.557), and Europeans (0.559). Analysis of genetic distances demonstrates continental clustering with high bootstrap support and large genetic distances between populations within Africa and between African and non-African groups. The pattern we observe is consistent with a bottleneck in non-African populations. Fst analysis indicates that 14.1% of *Alu* diversity occurs between the three continental groups of Africans, Asians, and Europeans. The Fst within each major group is 0.042, 0.021, 0.010, 0.021, and 0.045 for Africans, Asians, Europeans, Indians, and tribal Indians, respectively. Short tandem repeat polymorphisms (STRPs) within 50 kb of 12 different *Alu* loci were also analyzed. The STRP allele size variance ratio is highest in Africans (1.33), intermediate in Europeans (0.91), and lowest in Asians (0.76). STRP heterozygosity is highest in Africans (0.84) and lower in Europeans (0.77) and Asians (0.73). These results indicate a decrease in the number of STRP alleles and STRP diversity with increasing distance from Africa. Haplotypes of *Alu* markers and neighboring STRPs were constructed. This allowed us to date each *Alu* insertion event and to assess ancestral (*Alu* absent) versus derived (*Alu* present) haplotypes. Ancestral haplotypes generally demonstrate high levels of STRP variability, especially in African populations. Derived haplotypes marked by low-frequency *Alu* insertions have low STRP allele variability while older, high-frequency *Alu* haplotypes have higher STRP variability. The results are consistent with an out-of-Africa model of evolution and a genome-wide reduction in haplotype diversity signifying a bottleneck in populations leaving Africa. (Support: NIH grant GM-59290, NSF grant SBR-9818215).

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Patterns of DNA Variability in Human Genes. *J.C. Stephens, J.A. Schneider, M. Pungliya, J. Choi, R. Jiang, X. Sun, B.A. Salisbury, R.S. Judson, G.F. Vovis.* Genaisance Pharmaceuticals, New Haven, CT.

We have investigated the DNA-based variation (both SNPS and haplotypes) for nearly 4,000 human genes. In addition, we characterized how this variation is distributed in a number of biologically and clinically important ways. First, we determined: the pattern of how SNPs are distributed within various functional regions of a gene; the frequency with which these SNPs are found in different populations; the pattern of the sequences in which these SNPs occurred; and how these human SNPs compared to the corresponding sequence of a chimpanzee. Second, we determined how these SNPs varied among 82 unrelated individuals: 20 African-Americans, 20 East Asians, 21 European-Americans, 18 Hispanic-Latinos and 3 Native Americans. In particular, we examined patterns of SNP and haplotype sharing among the four larger population samples. Third, we determined the patterns of linkage disequilibrium among these SNPs and, hence, defined the haplotype variability of each gene, which varied substantially among the four populations. An understanding of these patterns of variability within and among genes is a fundamental prerequisite for connecting important clinical variability (e.g., genetic disease or disease susceptibility and variable drug response) to the DNA variability of human genes.

Contrasting patterns of variation in *CCR5* in humans and chimps. *S. Wooding*¹, *S. Mummidi*^{2,3}, *E. Gonzalez*^{2,3}, *S.S. Ahuja*^{2,3}, *D.M. Dunn*^{2,3}, *A.C. Stone*⁴, *L.B. Jorde*¹, *R.B. Weiss*¹, *M.J. Bamshad*¹. 1) Department of Human Genetics, University of Utah, Salt Lake City, UT; 2) Veterans' Administration Research Center, South Texas Health Care System, San Antonio, TX; 3) University of Texas Health Science Center, San Antonio, TX; 4) Department of Anthropology, University of New Mexico, Albuquerque, NM.

CCR5 encodes a cell surface chemokine receptor molecule that serves as a principal coreceptor, with *CD4*, for HIV-1 and SIV. Varied HIV-1 susceptibility has been associated with polymorphisms in the 5' cis-regulatory region of *CCR5*, suggesting that it may have been a target of natural selection. We characterized sequence variation in this region in 400 chromosomes from worldwide human populations and 50 chromosomes from 3 subspecies of chimpanzees. In humans, variation was substantially higher than expected and characterized by an excess of intermediate-frequency alleles. A genealogy of *CCR5* haplotypes had deep branch lengths despite markedly little differentiation among populations. This suggested a deviation from neutrality not accounted for by population structure, and was confirmed by tests for natural selection. This indicates that the pattern of genetic variation in *CCR5* has been shaped by balancing selection. The pattern of variation in chimpanzees was markedly different. For chimpanzees, genetic variation was lower than that observed at other autosomal loci, and in contrast to other loci, it was lower than diversity estimates for human *CCR5*. Moreover, variation in chimpanzees was characterized by a significant excess of rare variants. A genealogy of *CCR5* haplotypes had a star-like pattern, the central node of which represented the only haplotype shared by all 3 subspecies. This indicates that the pattern of genetic variation in *CCR5* in chimpanzees may have been affected by a selective sweep. This pattern may reflect an older adaptive response of chimpanzees to pathogens such as SIV. Analysis of patterns of variation between subspecies of chimpanzees infected with SIV vs. those in which SIV has never been reported should provide further insights.

Association/Linkage and Hardy-Weinberg Equilibrium at the NAT2 locus in Kindreds with Colorectal

Neoplasia. *D. Daley*¹, *R.C. Elston*^{1,4}, *S. Iyengar*¹, *S.D. Markowitz*^{2,4}, *G.L. Wiesner*^{3,4,5}. 1) Departments of Epidemiology and Biostatistics;; 2) Medicine; 3) Genetics: Case Western Reserve University, Cleveland, OH; 4) Ireland Comprehensive Cancer Center; 5) Western Reserve University and University Hospitals of Cleveland.

We recently showed linkage between colon neoplasia and the NAT1/2 locus in 56 severely affected sibships from the Colon Neoplasia Sibling Study (Daley et al, 2002) using the affected sibpair method and Haseman-Elston regression analysis. The sibships were selected on the basis of having at least two sibs affected with an advanced histology and/or early-onset colon neoplasia diagnosed by age 51. Based on our excess allele sharing at NAT2, we hypothesized that specific alleles are associated with disease. If so, associations between these alleles and a departure from HWE would be expected. NAT2 was genotyped at 9 different RFLP sites within the coding region of the gene in the 56 kindreds and parental origin of the alleles could be determined for 12 cases and 36 pseudo-controls. Pseudo-case control conditional logistic regression was performed to examine the relative risks (RR) at each RFLP site. The most significant results were found at the T341C and C481T sites, which code for the NAT2*5A/B/C alleles, previous studies have suggested an association with colon cancer. 11 of 12 cases were heterozygotes at both sites, vs. 13 of 36 pseudo-controls (RR of the T341C and C481T heterozygotes, $p < .00001$). Due to the genotypic dependence of full siblings, standard random sample methods cannot be used to test for HWE in family-based studies. Thus, we developed a sibpair method (SP-HWE) that uses all of the sibpair information in the 56 kindreds to test for HWE at each diallelic RFLP site and overall for the multiallelic NAT2 locus. Significant evidence for departure from HWE was identified for T341C and C481T, supporting an association between these alleles and colon neoplasia. Our method has independently identified a class of colon neoplasia susceptibility alleles and may be used for similar investigations in family-based studies. (Funded by American Cancer Soc PI DD).

Correlation between traits increases the power of linkage detection. *M.d. Andrade*¹, *C. Olswold*¹, *S.L.R. Kardia*², *E. Boerwinkle*³, *S.T. Turner*⁴. 1) Div Biostatistics, Mayo Clinic, Rochester, MN; 2) Dep Human Genetics, University of Michigan, Ann Arbor, MI; 3) Human Genetics, University of Texas Health Science Center, Houston, TX; 4) Div Hypertension, Mayo Clinic, Rochester, MN.

Genome-wide linkage analyses for complex traits such as blood pressure (BP) level and diagnostic category are challenging. Although these traits aggregate in families, they usually do not segregate in a Mendelian fashion, since multiple genes, each of which is polymorphic and has small-to-moderate effects, influence them. Since elevated BP level is the underlying measure of hypertension, several studies have aimed to identify genes that contribute to interindividual variation in BP level. However, none of these studies considered the combined effects on multivariate phenotypes such as systolic blood pressure (SBP) and diastolic blood pressure (DBP) or a measure of blood pressure (e.g., SBP or DBP) and another correlated trait (e.g., body weight). We have performed such multivariate linkage analyses for SBP and DBP using the Rochester Family Heart Study data. Traits that are genetically correlated were included in the analyses. We define polygenic genetic correlation as a measure of correlation between genes simultaneously influencing two traits (the higher the correlation, the more genes the traits share in common). We observed an increase in the bivariate LOD values for SPB and DBP compared to their separate univariate LOD values (an increase varying from 0.5% to 500% throughout the whole genome). These results confirm what we have observed previously in simulated data -- i.e., the power of detecting linkage is greater for multivariate correlated traits than for individual traits analyzed one-at-a-time. Thus, by using multiple traits that are correlated with BP level will provide an enhanced ability to identify responsible genetic factors, which in turn will lead to better understanding the genetic basis of this complex multifactorial phenotype and serve as a basis for the development of more comprehensive prevention and treatment strategies.

Association of an IL-1RN polymorphism with skin prick test positivity in Sheffield asthma families. *G. Ng Man Kwong¹, A.S. Rigby², A.R. Proctor¹, S.P. Ellis¹, R.A. Primhak², M.K.B. Whyte¹.* 1) Respiratory Medicine Unit, Royal Hallamshire Hospital, Sheffield, S Yorkshire, UK; 2) Sheffield Children's Hospital, University of Sheffield, Sheffield, UK.

Background and methods: Skin prick test positivity (SPT+) has been used as an intermediate phenotype for allergic asthma and atopy. In this study, we investigated the relationship between polymorphisms in the interleukin-1 gene cluster and SPT+ to multiple allergens (grass, house dust mite, cat, dog, cockroach, alternaria). Polymorphisms of IL-1 receptor antagonist (IL-1RN) (+2018), IL-1B (-511), IL-1B (-3954), IL-1A (+4845) were studied. Allelic frequencies were determined by 5 nuclease assays (Taqman allelic discrimination test) on DNA obtained from 97 families (421 individuals) recruited with two or more asthmatic siblings. Data was analysed using the transmission disequilibrium test (TDT). Results: A significant association was observed between SPT+ and IL-1RN allele 2.

Genotype		Not Transmitted	Transmitted	TDT Test	p value
	Transmitted	Allele 1	Allele 2		
IL-1RN	Allele 1	128	45	5.83	0.016
	Allele 2	71	22		

When this genotype was examined further we found (1) that the data could be replicated for at least 3 different aeroallergens and (2) there was a dose-response effect for 2 SPT+ ($p=0.035$) and 3 SPT+ ($p=0.014$). Associations with the other polymorphisms were not significant. Conclusions: Our findings suggest that a polymorphism in IL-1RN may be important in determining atopy. Abstract funded by GlaxoSmithKline.

Affected-Relative-Pair Linkage Analysis with Covariates for Hypertension in NHLBI Family Blood Pressure Program (FBPP). *X. Wu¹, B. Thiel², S. Kardiac³, D.C. Rao⁴, M. Province⁴, K. Ranade⁵, X. Zhu¹, A. Luke¹, D. Kan¹, R. Cooper¹.* 1) Dept Preventive Med/Epidemiol, Loyola Univ, Chicago, Maywood, IL; 2) Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH; 3) Department of Epidemiology, University of Michigan, Ann Arbor, MI; 4) Division of Biostatistics, Washington University of School of Medicine, St. Louis, MO; 5) Department of Genetics, Stanford University School of Medicine, Stanford, CA.

An important factor affecting the power to detect linkage in complex diseases studies is locus heterogeneity, which can weaken or even eliminate evidence for linkage that is present only in a subset of families. In a genome scan of adult hypertension in the FBPP study, we used a model-free linkage analysis with covariates developed by Olson (1999) and extended by Goddard et al. (2001). This method is a general conditional-logistic model using affected relative pairs. Discrete or quantitative covariates included in the model increase power to detect linkage when the covariate measures differences, between families, that are important to locus heterogeneity. We performed linkage analyses in four ethnic groups (white, black, Mexican American and Asian) within four networks in FBPP separately, followed by the analyses in pooled white and black samples. Two covariates, age and body mass index (BMI), were selected because they are significantly correlated with hypertension status. Without incorporating covariates, only one region showed suggestive evidence of linkage with $P < 0.001$ ($LOD > 2.08$) in individual genome scans (marker D18S481, $LOD = 2.23$). Two more regions showed suggestive evidence of linkage ($P < 0.001$) when age or BMI was incorporated as covariate in the analysis: 2p21(marker D2S1788, $LOD = 3.09$, covariate = BMI) and 3q22 (marker D3S1744, $LOD = 3.16$, covariate = age). In the pooled black samples, a new region on chromosome 8 (marker D8S1477, $LOD = 2.84$) showed evidence of linkage in an analysis without covariates. Our results suggest that incorporating appropriate covariates may increase the power to detect linkage in complex diseases mapping.

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Within haplotype block: which SNPs to choose? *Q. Huang, E. Boerwinkle.* Human Genetics Ctr, Univ Texas, Houston, Houston, TX.

Linkage disequilibrium (LD) between single nucleotide polymorphisms (SNPs) caused reduced number of haplotypes defined by these SNPs. Genomic regions with limited haplotype diversity, so called haplotype blocks, have been characterized in different chromosomes by several recent studies. With the knowledge of underlying haplotype structure of the human genome, certain subset of SNPs could be typed to represent the haplotype blocks and haplotype analysis could be carried out to test the association of genomic segments with disease. But which SNPs comprise the best subset of SNPs that capture most of the information of the haplotype blocks remains unclear. Here we test the association between haplotypes derived from different subset of SNPs and disease endpoint assuming simple case/control sampling design. LD between these SNPs was also characterized. The analysis was carried out on simulated dataset from Genetic Workshop12 (GAW12), where MG6 is one of the seven major genes that contribute to a complex disease, and the location of the casual mutation is already known. Results showed that the causal mutation is not necessarily needed to detect the association. Haplotype analysis could always detect the association, but at least one of the SNPs should be in strong LD with the causal mutation. This suggests that haplotypes comprised by SNPs with low pair-wise LD capture more information than haplotypes comprised by SNPs with high pair-wise LD. Detailed LD information within haplotype blocks could help us determine which subset of SNPs should be chosen to be typed to represent the haplotype blocks.

The transcriptional factor LBP-1c/CP2/LSF gene in chromosome 12q13 is not associated with asthma in two independent populations. *A. Zambelli-Weiner¹, D.H. Farkas², V. Chan³, R.A. Mathias¹, V. Casolaro¹, K.C. Barnes¹.*
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CP2, also known as leader-binding protein (LBP)-1c or late SV40 factor (LSF), is a 502-aa nuclear protein shown to regulate transcription of a number of viral and cellular genes including HIV-1 and IL-4. Recent studies showed increased CP2 expression in atopic subjects and in a subgroup of Alzheimer patients. This has been shown to be concomitant to a non-coding polymorphism (GA) in the CP2 3' untranslated region (UTR) at position #2236, a region possibly regulating RNA stability. Because we have previously observed evidence for linkage to markers flanking CP2, and because of the potential role of CP2 in the allergic diathesis, we investigated whether the presence of the 3' single nucleotide polymorphism [SNP] (G2236A) and a promoter SNP (G-310T) was associated with asthma or atopy in both an Afro-Caribbean population (N=571) enriched for atopic asthma and Tangier Island, a complete Caucasian isolate (N=295). An electronic detection platform using DNA chip technology (eSensorTM; Motorola Life Sciences) has been developed for detection of specific DNA sequences for use in molecular diagnosis. In this study we applied this arrayed electrochemical detection platform for simultaneous detection of the two CP2 SNPs. There were no significant associations with asthma for either of the two CP2 SNPs in both populations. Interestingly, the A allele in the 3' SNP, which was reported to have a protective effect for Alzheimers disease in three independent Caucasian populations, was rare in both the Barbados (GA genotype=2.28%; AA genotype=0%) and Tangier Island (GA genotype=10.51%; AA genotype=0%) populations. The GG genotype conferred slightly higher total serum IgE concentrations in the Barbados parent-offspring trios (GG genotype=549.5 ng/mL vs. GA genotype=407.4 ng/mL) but was not significant. In summary, our results do not support CP2 as a candidate gene/risk factor for asthma, although its potential role in the allergic diathesis is still unclear.

Fetal and infant growth rate: genetic and environmental correlations. *E.W. Demerath, S.A. Czerwinski, B. Towne, S.S. Guo, W.C. Chumlea, R.M. Siervogel.* Lifespan Health Research Center, Wright State Univ Sch Med, Dayton, OH.

It has been recently shown that children who grow slowly in utero, but grow rapidly during infancy, appear to be at elevated risk of a number of chronic diseases in adulthood, including diabetes and coronary heart disease. The association between fetal growth retardation and postnatal "catch-up" is usually assumed to be due to the cessation (at birth) of a restrictive intrauterine environment, but the genetic associations amongst these traits are still unknown. In this study, we sought to estimate the degree to which fetal growth and subsequent infant growth rate are influenced by a common set of genetic and environmental factors. The study sample included 537 infants in the Fels Longitudinal Study (N=276 males/261 females) who were followed for two years. Weight and length were measured at birth, and length was measured at 3 months, 6 months, 9 months, 12 months, 18 months and 24 months. We modeled the serial data using a 3rd degree polynomial model, and estimated the growth rate and acceleration for length. Using a maximum likelihood variance components approach, we estimated the heritability (\pm stderr) of the traits of interest as follows: Birth Weight: $h^2=0.79\pm0.09$; Birth Length: $h^2=0.78\pm0.10$; Inf. Length Rate: $h^2=0.34\pm0.10$; and Inf. Length Acceleration: $h^2=0.34\pm0.11$ (all significant $p<0.001$). We then tested the genetic and environmental correlations among these traits. As expected we found evidence for substantial shared genetic effects influencing Birth Weight and Birth Length ($r_G=0.74\pm0.12$). Interestingly, we found no genetic correlation, but rather a moderate environmental correlation between Birth Length and Infant Length Rate ($r_G=-0.54\pm0.18$). The latter finding indicates that the environmental factor/s involved in fetal growth retardation are also involved in rapid infant growth rate. These findings demonstrate the large effect of genes on fetal and infant growth rate and bring into question the notion that "catch-up" growth is recovery from intrauterine restriction. Rather, they suggest that other environmental factors common to the pre- and post-natal environment may explain the association of low birth weight and high infant growth rate.

Effects of heredity and helminthic infections on the growth of children in rural Nepal. *B. Towne¹, S.A.*

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Growth is influenced by both genetic and environmental factors, but quantifying their respective contributions is not possible without measures of growth from related children and measures of specific environmental factors known to impact growth. The Jiri Growth Study was recently initiated in collaboration with the Jiri Helminth Project to examine the growth and development of children from the Jirel ethnic group in eastern Nepal where parasitic gastrointestinal infections are endemic. Because helminthic infections negatively impact child health, the goal of the Jiri Growth Study is to elucidate genetic influences on the growth and development of Jirel children taking into account helminthic infection status. In these analyses we used a maximum likelihood method for pedigree data to estimate the heritability of the stature of Jirel boys and girls, while simultaneously modeling the effects of prior history of infection with roundworms, hookworms, or whipworms on stature. Analyses were conducted on data from 1,046 children (517 boys and 529 girls) aged 3 to 18 years, most of whom are from one very large extended pedigree. The heritability of stature in this study sample was highly significant at 0.87, and significant negative effects of roundworm and hookworm infection histories on stature also were found. For example, after taking into account genetic sources of variation in stature, Jirel children with histories of roundworm or hookworm infections are some 1 - 2 cm shorter than children with no prior history of infection. These findings show stature during childhood to be highly heritable, even in this heavily parasitized population, and that in the Jirel population a significant portion of the residual phenotypic variance in childhood stature can be attributed to specific helminthic infections.

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Reduced haplotype diversity at the gene encoding Hepatocyte Nuclear Factor 1 alpha. *T. Frayling, K. Thomson, K. Colclough, S. Ellard, A.T. Hattersley.* Dept Diabetes & Vascular Med., Exeter Univ, Exeter, Devon, England.

The gene encoding Hepatocyte Nuclear Factor 1 alpha (TCF1) is an important diabetes gene. Mutations cause the single gene disorder Maturity onset diabetes of the young (MODY) and in one isolated population a common variant predisposes to type 2 diabetes. It also lies in a region of chromosome 12 linked to type 2 diabetes in some genome wide scans. It is not known whether common variants in TCF1 predispose to type 2 diabetes in other populations. Recent studies indicate that due to linkage disequilibrium (LD) there is greatly reduced haplotype diversity across genes when assessing all common (minor allele frequencies (MAF) > 5%) variation. This will potentially facilitate a more robust assessment of the role of gene variation in disease association studies than previously used. We aimed to assess the variation across TCF1 and the resulting haplotype diversity. Sequencing the coding region and intron-exon boundaries in 71 unrelated diabetic (including 14 with a MODY mutation) subjects identified 13 single nucleotide polymorphisms (SNPs) (6 coding) with MAFs ranging from 3 to 37 %, 12 of which had MAFs > 5%. The 2 outermost SNPs are ~22 kbp apart and occur in the first exon and intron/exon boundary of the last exon. A pseudo-Bayesian algorithm, as implemented in the program PHASE was used to estimate haplotype frequencies. Five haplotypes were present at frequencies > 5% and accounted for 82% of all haplotypes ($p < 0.00001$ vs expected). These estimates may be biased towards haplotypes more frequent in diabetes/MODY subjects. There was no evidence that TCF1 mutations occur on certain haplotypes ($p = 0.13$) but analysis of additional subjects with TCF1 mutations and their family members is needed to assess this more accurately. Three SNPs can be used to capture all common haplotype information across the TCF1 gene in association studies. In conclusion we have shown for the first time that the diabetes gene TCF1 exhibits greatly reduced haplotype diversity. This provides further support for the hypothesis that common variation across genes occurs on a limited number of common haplotypes.

Y-Chromosomal Microsatellite Haplotypes in Populations from the Venezuelan North Western Region. *L. Borjas-Fajardo, L. Pineda Bernal, M.A. Sánchez, E. Fernández, W. Zabala, C. Castillo, L.A. Socca, A. Rojas.* Molecular Genetic Laboratory. Medical Genetic Unit. University of Zulia. Venezuela.

The first inhabitants of Zulia State, North Western region of Venezuela, were Arawaco, Caribe and Chibcha natives. Currently this region is a combination of diverse populations, which are the result of a mixture process between the native populations and immigrants, for which reason it is relevant to know and clarify the genetic structure of them. 160 genetically unrelated males were analyzed for four Y-STRs: DYS19, DYS390, DYS391 and DYS393. The samples were obtained from three populations: Maracaibo (100), Wayuu (30) and Island of Toas (30). DNA samples were extracted by conventional techniques, Y-STRs were obtained individually through PCR and samples were characterized on electrophoresis polyacrylamide gels and visualized using silver staining. Allele designation for all loci was ensured using loci-specific allelic ladders, and results were processed employing the Arlequin program. Allele frequencies of each system were compared with the mean frequencies reported in the literature for Europeans, Asians, Africans and Amerindians. It was observed that there were higher levels of gene diversity in Island of Toas and Maracaibo than in Wayuu, except for the locus DYS393 and very markedly for the locus DYS390. Fst values showed significant differences only between Maracaibo and Wayuu. Haplotype diversity was similar across the three populations. The most frequent haplotypes in Wayuu included allele markers typical of Amerindians, whilst the most frequent in Maracaibo and Island of Toas included alleles typical of Europeans. The three populations shared only three haplotypes. The most frequent haplotype found in Maracaibo and Island of Toas is present only once in Wayuu. The two most frequent haplotypes in Wayuu are absent in Island of Toas and one of them is present once in Maracaibo. The exclusive haplotypes of each population showed low levels of frequency except in Wayuu, where the haplotype 13-23-11-14 is the most frequent. It is necessary to increase the number of Y-STR markers and include some SNP, in order to study the origins of these populations in more depth.

Haplotype frequencies in the beta fibrinogen gene. *Y. Berthier-Schaad*^{1,2}, *Y. Liu*¹, *N. Fink*¹, *M.J. Klag*¹, *J. Coresh*¹, *M.W. Smith*³. 1) Johns Hopkins Med. Insts., Baltimore, MD; 2) Laboratory of Genomic Diversity, NCI, Frederick, MD; 3) Basic Research Program, SAIC, NCI, Frederick, MD.

Fibrinogen levels have been implicated as an important risk factor for cardiovascular diseases (CVD). In some studies, several nucleotide polymorphisms (SNPs) located in the promoter and the coding regions of the beta fibrinogen gene have been associated with increased fibrinogen levels and CVD. The difficulty in associating genotype and phenotype has led to the recognition that haplotype-based methods represent a promising approach to a better understanding of the genetic basis of common diseases and led to the human haplotype map project. As a step in the direction of understanding haplotypes at the beta fibrinogen gene, seven SNPs, five in the promoter region (-1420G/A, -854G/A, -455G/A, -249C/T, -148C/T) and two in the coding regions (345C/T in exon 7, 448G/A in exon 8), were analyzed. We obtained 1186 haplotypes from a population of 784 incident dialysis patients of the CHOICE cohort. Haplotypes and their frequencies were estimated using the PHASE program. Six major haplotypes were observed: GGGCCCG (47%, 67%); GAGCCCG (13%, 9%); GGGTCCG (21%, 7%); AGACTTA (16%, 5%) in European Americans and African Americans respectively. Two haplotypes GGGCCCA and AGGCTCG were present in African Americans only with a frequency of 5% each. The fibrinogen haplotypes can be separated into three natural groups where each group only differs stepwise from the others by one nucleotide, a C to T mutation on one of the DNA strands. A haplotype tree can be generated using GGGCCCG as the ancestral haplotype. These findings are consistent with recent analyses of haplotype blocks showing that 6 to 8 common SNPs suffice to identify common haplotypes in a region inherited without significant historic recombination. A greater diversity of haplotypes was also found in African Americans. Determination of these haplotypes will facilitate their evaluation relative to CVD and fibrinogen levels in patient cohorts. Funded in part by RO1 L62985, AHA EI01-40197ND, & DHHS#NO1-CO-12400.

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Investigation of MtDNA haplogroups and mitochondrial disorders(Report from Iran). *M. Houshmand, F. Sharifpanah, A. AleMohamad, M. Rahmani, F. Babrzadeh, M. Vakilian, S. Joughehdoust, M.H. Sanati.* Medical Genetics Unit, National Research Center for Genetic Engineering and Biotechnology Tehran, Iran.

Analysis of the mtDNA haplogroups is, however, not only restricted to the studies of human origin and evolution. It is also very important for studies of human pathologies. Indeed, recent studies have shown that mtDNA haplogroups can play an important role in modulating disease/phenotype expression. Such as Multiple Sclerosis (MS) (haplotype K and J); Leber Hereditary Optic Neuropathy (LHON) (haplogroup J). The early history of man in Iran goes back well beyond the Neolithic period, it begins to more interesting around 6000 BC, when people began to domesticate animals and plant wheat and barley. The number of settled communities increased particularly in the eastern Zagros mountains. For analysis of MtDNA haplogroup in Iranian population we started with 11778, 3460, 14484, 14459 mutations in 24 LHON and 50 MS affected patients. We detect 11778 mutation from 5 LHON patients and no association found between MS and these mutations. Haplogroup J is only about 9% of general European population but it was found in 37% of LHON patients harboring the LHON mutation. *HinfI* restriction site at np 16065 (haplogroup J) analyzed in our 5 screened LHON patients (positive for 11778) and 20 MS patients. Haplogroup B defined by the 9np COXII-tRNA Lys deletion that is common throughout central and southern Asia and is prominent in coastal Asian population but was absent from all 152 Iranian that we analysed. (Arab, Turk, Kurd, Lor, Baluchi groups) Other prominent Asian haplogroup G and also found in the European populations was detected in one Friedreich Ataxia patient. More investigation is needed to identify that there is any relation between Friedreich Ataxia and haplogroup G or not.

Endothelial constitutive nitric oxide synthase gene polymorphism in Korean population. *S. Han, Y. Jung, J. Oh, M. Oh.* Biology, Cheju National University, Cheju, Cheju, Republic of Korea.

Endothelium-dependent vasodilatation is mediated by nitric oxide formed by constitutively expressed endothelial nitric oxide synthase (eNOS). Recently, three point mutations, variable numbers of tandem repeats (VNTR), microsatellite (CA)_n polymorphism, and Glu298Asp in the endothelial constitutive nitric oxide synthase gene have been reported. In addition, the significant associations of the *eNOS4b/a* polymorphism in intron 4 VNTR and Glu298Asp (G1917T) with coronary artery disease, essential hypertension, renal diseases and Alzheimer's disease have been reported in some populations. We analyzed the VNTR of *eNOS* intron 4 using polymerase chain reaction, polyacrylamide gel electrophoresis, and DNA sequencing. We inspected the polymorphism of the *eNOS* intron 4 in 228 healthy individuals of Korean population. The detected alleles were *b* and *a*, keeping four or five tandem 27-bp repeats, respectively. The genotype frequencies of *eNOS4b/b*, *eNOS4b/a*, and *eNOS4a/a* were 79.82%, 18.86%, and 1.32%, respectively. VNTR of intron 4 showed also polymorphism in Korean population, but allele *c* containing six tandem repeats was not found. On the basis of these results, we will investigate the associations between the polymorphisms of *eNOS* gene and related diseases.

Aldehyde dehydrogenase 2 (ALDH2): Strong LD around the world. H. OOTA, A.J. PAKSTIS, J.R. KIDD, K.K. KIDD. Dept Genetics, Yale U, New Haven, CT.

Aldehyde dehydrogenase 2 (*ALDH2*) is a major enzyme in acetaldehyde oxidation related to alcohol metabolism. The catalytic deficiency of human *ALDH2* has been well known to be present only in East Asia, which is caused by a substitution (G to A) inducing Glu to Lys amino acid change in exon12 (Ex12c). We have typed five SNPs and one STRP for 2006 individuals from 38 worldwide populations. The five SNPs have been selected to cover the *ALDH2* locus uniformly. Four non-coding SNPs are located approximately 40, 30, 20 and 10kb upstream of Ex12c, whereas the STRP site, D12S1344 (CA)_n, is 83kb downstream of this functional variant. Analyses are complete for 33 of the 38 populations.

The polymorphism in intron1 (30kb from Ex12c) is found in Ethiopian Jews and most non-African populations at moderate to high heterozygosities, but was not seen in many west African and central African populations and only rarely in other Africans. Thus, not considering the functional Ex12c variant, only four of 16 possible haplotypes account for almost all chromosomes in all populations. Only three of those haplotypes are common in African populations. The functional variant, Ex12c, occurs on one of those to define a fifth common haplotype in East Asians. The frequencies of those common haplotypes are quite similar within regions of the world but differ considerably from region to region. The STRP shows LD in Africa with different allelic distributions on the three common *ALDH2* haplotypes. The LD is even stronger in non-African populations with more reduced distributions of STRP alleles associated with the four common haplotypes. Thus, LD is strong and highly significant in all parts of the world extending across 120kb.

The *F_{st}* values in 32 populations for the most upstream SNP and Ex12c were .30, and .26, respectively, about two SD above the average of 94 reference sites on other chromosomes (.14). These results suggest that the *ALDH2* locus might have experienced selection as modern human populations evolved. [Supported in part by NIH grant AA09379].

Mitochondrial DNA Diversity Among the Five Civilized Tribes of Oklahoma. *C.A. Jantzen, S. Stevens.* Latta High School, Ada, OK.

To show the feasibility of assessing mtDNA diversity in close Native American tribes, hair follicle DNA was extracted from 20 individuals of each of 5 tribes, after gaining science fair IRB approval, chiefs' permission, and individual informed consent. We only took individuals of strict maternal descent from a full blooded woman of one of the Five Civilized Tribes, who were forced to migrate from the southeast US in the 1820s. 440 nucleotides in the HVI region were amplified; Cold Spring Harbor Laboratories sequenced the fragments. The numbers of single nucleotide differences between all possible pairs of subjects within a tribe were averaged ("intratribal differences"); the intertribal difference was similar mean for all possible pairs of subjects, taken one from each tribe. The smallest differences within single tribes was in the Chickasaw; the most, in the Cherokee; findings consistent with other linguistic and historical evidence. Intertribal differences were least between Creeks and Choctaws and greatest between Creeks and Cherokees; these differences are unexplained.

Chickasaws	Choctaws	Muscogee	Seminoles	Cherokees
Intratribal 4.4	Intratribal 5.5	Intratribal 6.1	Intratribal 11.0	Intratribal 13.6
Intertribal 9.4	Intertribal 8.8	Intertribal 10.6	Intertribal 10.1	Intertribal 13.2
Choctaw 7.8	Chickasaw 7.8	Chickasaw 8.7	Chickasaw 8.3	Chickasaw 12.7
Muscogee 8.7	Muscogee 6.9	Choctaw 6.9	Choctaw 7.6	Choctaw 11.8
Seminole 8.3	Seminole 7.6	Seminole 10.9	Muscogee 10.9	Muscogee 14.7
Cherokee 12.7	Cherokee 11.9	Cherokee 14.7	Cherokee 13.5	Seminole 13.5

Ethnic differences in polymorphisms in GCLC, the gene that encodes the catalytic subunit of glutamate cysteine ligase. A.S. Willis¹, M.L. Freeman², S.R. Summar³, S.M. Williams⁴, E. Dawson⁵, F. Barr⁶, M.L. Summar³. 1) Molecular Physiology and Biophysics, Vanderbilt University Medical Center, Nashville, TN; 2) Radiation Oncology, Vanderbilt University Medical Center, Nashville, TN; 3) Pediatrics/Genetics, Vanderbilt University Medical Center, Nashville, TN; 4) Meharry Medical College, Nashville, TN; 5) BioVentures, Inc., Murfreesboro, TN; 6) Pediatric Cardiology, Vanderbilt University Medical Center, Nashville, TN.

GCLC is the gene that encodes the catalytic subunit of glutamate cysteine ligase, a heterodimeric enzyme that catalyzes the first step in *de novo* synthesis of glutathione, an abundant biomolecule. Glutathione plays roles in many cellular processes including the scavenging of free radicals to neutralize oxidative stress. The pathophysiology of several diseases, including neurodegenerative diseases, and progression of HIV infection, is thought to involve glutathione and oxidative stress. We have identified 11 polymorphisms in GCLC and determined allele frequencies in Caucasians and African Americans. Allele frequencies for 4 polymorphisms, C1384T, IVS12+83G/A, IVS12-21G/A, and 1974-1975insCAGC, differ between Caucasians and African Americans ($p < 0.05$). The allele frequencies for IVS12+83G/A and 1974-1975ins CAGC, vary widely between the two groups ($p < 0.001$). One polymorphism, the nonsynonymous C1384T, has been found only in individuals of African descent; the rare allele (T) has an allele frequency of 2% in African Americans and 5% in Africans from Ghana in western Africa. Proline 462, which is changed to a serine by the T allele, is conserved in a number of eukaryotic species, suggesting that this polymorphism may have functional significance.

Derivation of immortal cell lines from monospermic complete hydatidiform moles: A new resource for haplotype discovery. *V.P. Stanton Jr¹, W.C. Hahn², W. Wojcicki¹, J. Olson¹, S. Jasani³, P.-Y. Kwok⁴, U. Surti³.* 1) Variagenics, Cambridge, MA; 2) Dana-Farber Cancer Institute, Boston, MA; 3) Department of Pathology, University of Pittsburgh, Pittsburgh, PA and Magee-Womens Research Institute, Pittsburgh PA; 4) University of California, San Francisco, CA.

Haplotypes are expected to provide a more powerful system for allele classification than genotypes, and to lead to improved methods for genetic association studies. The only robust methods for haplotype discovery are pedigree analysis and sequencing cloned DNA. Both approaches are inefficient for determining the haplotypes of specific genes in large numbers of subjects. Monospermic complete hydatidiform mole (MCHM) is a rare human tumor formed by the fertilization of an anuclear ovum by a single sperm with subsequent duplication of the paternal chromosomes, yielding a diploid cell which is completely homozygous. DNA sequencing or genotyping of MCHMs yields haplotypes. Exploitation of MCHMs for haplotype identification has been constrained by the difficulty of obtaining material, and by the limited amount of DNA that can be obtained from each specimen. We report that high efficiency introduction of the telomerase catalytic subunit, hTERT, immortalizes human MCHMs.. DNA from eight immortalized MCHMs was proven to be homozygous by DNA sequencing and genotyping. One of us (US) had the foresight to collect primary cultures of MCHMs for the last 20 years. Application of the TERT immortalization method will lead to the production of large numbers of completely homozygous cell lines, suitable for efficient identification of both common and rare haplotypes in genes of interest.

A coalescent estimate of ancestry informative markers in the genome. *C.L. Pfaff, J.C. Long.* University of Michigan Medical School, Ann Arbor, MI.

Admixture between genetically distinct populations can create non-random allelic associations (linkage disequilibrium) that can be used to map genes (Admixture Mapping). In order to effectively perform an admixture mapping study, it is important to identify a panel of Ancestry Informative Markers (AIMs), for which the allele frequencies in the founding populations are very different. The prevalence of AIMs in the genome is unknown. We have developed a coalescent program to simulate population divergence and microsatellite evolution using a stepwise mutation model in order to estimate the proportion of ancestry informative microsatellite loci in the genome. For each set of simulations, the effective population sizes and mutation rates were set according to the observed variance in allele size. Results show that, with a divergence time of 4000 generations ago and simulated founding populations with effective sizes of 10,000 and 5000 (approximating African and European effective sizes), a relatively large proportion of microsatellite loci demonstrate ancestry informative differences in allele frequency. The difference in allele frequency between populations is measured as d_c , which is calculated as the sum of the absolute values of the allele frequency differences for each allele size, divided by 2. On average, 43% of simulated microsatellite loci (out of 10,000 replications) exhibit a $d_c \geq 0.5$ when the mutation rate equals 0.0005 (mutations per generation) and 20% exhibit a $d_c \geq 0.5$ when the mutation rate equals 0.00005 (sample size = 50 observations from each population). For effective population sizes of 5000 and 2000 (approximating European and Native American effective sizes), an average of 70% of simulated loci exhibit a $d_c \geq 0.5$ when the mutation rate equals 0.0005 and 39% exhibit a $d_c \geq 0.5$ when the mutation rate equals 0.00005. These results are consistent with observational data for African American, Native American, and European American population samples and suggest that the abundance of AIMs in the human genome will make it possible to perform effective genome-wide admixture mapping studies. Efforts, therefore, should be made to identify AIMs throughout the genome and assemble a genome-wide admixture mapping panel.

Mannose-binding lectin and mtDNA Genotypes in Peruvian islanders (Quechua and Aymara) of the Lake

Titicaca. *J. Sandoval*¹, *H.O. Madsen*², *P. Garred*², *R. Fujita*¹. 1) Instituto de Genetica y Biologia Molecular, Facultad de Medicina, Universidad de San Martin de Porres, Alameda del Corregidor Cdra. 15, La Molina, Lima, PERU. rfujita@amauta.rcp.net.pe; 2) Dpt. of Clinical Immunology, National University Hospital, Copenhagen, Denmark.

Mannose-binding lectin (MBL) is a serum protein important for immunity, its defective alleles are associated with increased risk of infections and autoimmunity. The defective allele B (codon 54) was found in high frequency in one Amerindian population. We are interested in characterizing native Peruvian populations; thus, we analysed MBL genotypes (n=103) and mtDNA subtypes (n=144) in school children from islands on the Peruvian side of the Lake Titicaca: Taquile (T) and Amantani (Am) who speak Quechua; and Anapia (An) and Los Uros (U) who speak Aymara. We report the highest overall frequency (64%) of the allele B seen in any population so far. However, the distribution of the MBL genotypes differed highly significantly between Quechuas and Aymaras ($P < 0.0001$). Regarding mtDNA we found the highest scores of subtype B1 so far described (T = 100%, Am = 88.6%, An = 87.5%, and U = 75%). Other subtypes exist but in Los Uros it is noticeable the presence of A2 (17%), that is absent in the other islands. Our results indicate genetic differences between Quechua and Aymara populations. Due to the homogeneity of the genetic markers Taquile and Amantani seem to have been populated by a reduced founder group in agreement to one of the local oral traditions. The ethnical group "Uru" was assumed extinct from Peru; but our results suggest that contemporary Los Uros islanders still may have an "Uru" genetic background. Our preliminary studies in Amazonian populations suggest affinity with Los Uros, proposed decades ago by language and protein comparisons. The high frequency of the defective MBL allele B remains a very important topic to investigate in future epidemiological studies. Acknowledgement: Supported by a grant of CONCYTEC (Peru) and Universidad de San Martin de Porres, The Danish Council for Development Research, The Medical Research Council and The NOVO Nordisk Foundation.

Rapid purification of archival-quality DNA from large whole blood samples using a new rapid protocol. *J. Long¹, H.-W. Deng¹, Y. Zhang¹, L. Zhao¹, E. Hofer², C. Seemann², D. O'Brien².* 1) Creighton University, Omaha, NE; 2) Genra Systems, Minneapolis, MN.

Statement of Purpose: The efficient and effective purification of high quality DNA from large whole blood samples to support clinical studies has become increasingly important as laboratories around the world initiate studies designed to identify the underlying genetic contributions to the most common human diseases. The purification process has traditionally involved hazardous reagents and tedious protocols that compromise both the yield and quality of DNA. The purpose of this study was to identify a more rapid purification method that would provide high quality DNA suitable for archiving. We describe a new protocol, called PUREGENE EP(Enhanced Productivity), for use with the commercially available PUREGENE DNA Purification Kit, (Genra Systems, Inc.). This protocol dramatically reduces the total purification time without compromising yield or quality.

Methods: DNA was purified from replicate 10 mL human whole blood samples using the PUREGENE kit and either the standard PUREGENE protocol or the new PUREGENE EP protocol. The yield of DNA was determined by UV spectrophotometry. The quality of the purified DNA was assessed by analysis of the A_{260}/A_{280} ratio, gel electrophoresis, and performance in PCR amplifications.

Summary of results: Comparison of DNA from purified replicate human whole blood samples with the standard PUREGENE protocol and the PUREGENE EP protocol showed that the PUREGENE EP protocol was 50% faster while maintaining high DNA yield and quality. Yields were equivalent: ~ 300 g of DNA from 10mL samples from both the standard PUREGENE and the PUREGENE EP protocols. In addition, the quality of DNA isolated with the two methods, as measured by A_{260}/A_{280} ratios, DNA fragment size and performance in downstream assays, including PCR, was equivalent. These results suggest that the PUREGENE EP protocol provides effective and efficient purification of high quality DNA suitable for archiving while reducing the time required for purification.

Haplotype Map Of The Chromosome 17 CC Chemokine Gene Cluster. *W.S. Modi¹, M. Jamba¹, B. Roemer-Binns¹, C. Winkler¹, S. O'Brien².* 1) SAIC, NCI-Frederick Cancer Res, Frederick, MD; 2) Lab Genomic Diversity, NCI-Frederick Cancer Res, Frederick, MD.

Chemotactic cytokines mediate the directed migration of various cell types including monocytes, neutrophils, lymphocytes and megakaryocytes. These proteins are also involved in normal metabolic functions including T-cell development, signal transduction, and neuron differentiation. Further, HIV-1 uses chemokine receptors for cell entry, and several ligands are known to limit in vitro infection. Sixteen CC chemokine genes reside in three segments spanning 2 Mb at 17q21. These include: MCP-1, MCP-3, EOTAXIN, MCP-2, MCP-4, and I-309 in a 200 kb segment; RANTES, LEC, HCC-1, MPIF-1, HCC-2 PARC, MIP-1A and ACT-2 in a 250 kb segment; and LD78B and LAG-1 in a 40 kb segment. We have identified about 125 single nucleotide polymorphisms (SNPs) at varying densities covering each of the three segments. One hundred seventy-five individuals from each of three racial groups: European American, African American, and Chinese are being genotyped in order to determine allele and haplotype frequencies. Preliminary results indicate extensive linkage disequilibrium and reduced haplotype diversity exists within each segment. A complete definition of the haplotype structure in the entire 2 Mb region will enable targeted genotyping using representative SNPs in subsequent disease association studies. Funded in part by NCI, NIH Contract No. NO1-CO-124000.

Linkage Disequilibrium and Haplotype Structure of the Protein Tyrosine Phosphatase 1B (PTPN1) Gene Region on Human Chromosome 20. *M. Olivier, D. Savic, S. Brauer.* Human & Molecular Genetics Center, Department of Physiology, Medical College of Wisconsin, Milwaukee, WI.

Single nucleotide polymorphisms (SNPs) are of major interest for association studies in common human diseases. Factors that determine the usefulness of these markers are the extent of linkage disequilibrium (LD) between them, and the number of observed haplotypes in regions of significant LD.

In this study, we analyzed SNPs and the resulting haplotype patterns on human chromosome 20 surrounding the PTPN1 gene. The protein encoded by this gene, protein tyrosine phosphatase 1B, is associated with and regulates the activity of the insulin receptor. Several SNPs in this gene have been shown to be associated with type 2 diabetes, insulin resistance, and hypertension in humans. The gene PTPN1 is located on 20q13, and consists of ten exons covering 75 kb of genomic sequence.

We have previously identified SNPs in this genomic interval as part of our disease association studies. For the analysis of the LD and haplotype structure of the region, we selected 25 SNPs covering over 150 kb of genomic sequence around PTPN1. SNPs were genotyped on 90 unrelated individuals from different ethnic origin, and on ten Utah CEPH families (80 individuals) to obtain unambiguous haplotypes. SNPs in a region of over 100 kb covering the entire coding sequence of PTPN1 are in significant pairwise LD. Within this region, only four haplotypes with a frequency of greater than 5% can be identified. Alleles of SNPs that were previously identified in disease association studies are represented in these four major haplotypes. Data will be presented on the effects of different haplotypes on mRNA and protein levels of PTPN1 in human cells, and the potential functional relevance of these haplotypes for gene function.

Molecular genotyping and distribution of A¹, A², B, O¹, and O² alleles of the ABO locus in a Kuwaiti population.

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The present study is the first demonstration of an extensive variability linked to A¹, A², B, O¹ and O² alleles of the ABO locus in a Kuwaiti population. Genomic DNA from 200 unrelated healthy Kuwaiti blood donors was amplified using primers A10 and AB2. These primers amplify a 357-bp fragment, spanning nucleotides 385-742 of the ABO cDNA sequence. Diagnostic restriction enzyme digestions were carried out using either PvuII for the detection of the A² allele or BssHII for the detection of the O² allele. To distinguish the A allele from the O² allele, we chose to use nucleotide 526, which is a cytosine in the A (and O¹) alleles, but a guanine in the O² (and B) alleles. The base substitution in the O² and B alleles abolishes a BssHII restriction enzyme site found in the A and O¹ alleles. The typing at position 703 distinguishes the O² allele from the B allele. The base change at nucleotide 467 changes an MspI restriction enzyme site in the A¹ allele to a PvuII site in the A² allele. 13 genotypes arising from the major alleles of the ABO blood group; A¹, A², B, O¹ and O²; were distinguished in the Kuwaiti sample population. The calculated allele frequencies of A¹, A², B, O¹ and O² were 0.1579, 0.0199, 0.1566, 0.6243 and 0.0413, respectively. These frequency data of a Kuwaiti population may provide a useful additional information for forensic, population genetics, phylogenetics and evolution studies.

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The FKRP L276I change: High allele frequency and common cause of LGMD. *K. Gorni, M. Bakay, J. Devaney, E. Hoffman.* Res Ctr Genetic Medicine, Children's Ntl Medical Center, Washington, DC.

Limb-girdle muscular dystrophy (LGMD) is a disorder where weakness affects mainly the proximal limb-girdle musculature. To date, 15 genetically different types have been identified, which show great clinical and genetic heterogeneity. Autosomal dominant forms are very rare and generally less severe than recessive types. In 2001 Brockington et al used database mining to identify a gene related to the Fukuyama muscular dystrophy gene (FKRP), and showed association of mutations of this gene in both congenital muscular dystrophy and limb-girdle muscular dystrophy. Interestingly, a single C826A mutation (Leu276Ileu) was found homozygous in the large majority of LGMD mutation-positive changes, and this same change was found in 1:100 normal individuals, suggesting a high carrier rate for this change. We undertook a retrospective mutation analysis of 66 potential LGMD2I patients that had a previous inconclusive biopsy study. We choose to include in our sample a group of patients showing clinical features consistent with a limb-girdle muscular dystrophy and with a reduction of all proteins regularly screened in suspected muscular dystrophies. We performed first a restriction enzyme analysis confirming our cases with the sequence analysis, looking in both cases for the most frequently found mutation (the C826A, Leu276Ileu). We found 5 patients (9%) carrying this change, four as homozygotes (1 male and 3 females), and one compound heterozygote (male). This study confirms this single mutation as a relatively common cause of recessive LGMD, and suggests a founder effect and possible heterozygote advantage given the high frequency of this missense change. Antibody results are presented, as well as a non-PCR-based 3rd WAVE assay for rapid genotyping. We suggest that all Caucasians with LGMD should be screened for the FKRP L276I change. In addition, this data has ramifications for genetic counseling, given the relatively high rate of the change in the Caucasian population.

Genetic Variation in Beta Fibrinogen (FGB) and Plasma Fibrinogen Levels in Dialysis Patients: the CHOICE Study. *Y. Liu¹, Y. Berthier-Schaad², N. Fink¹, R.P. Tracy³, M.J. Klag¹, M.W. Smith⁴, J. Coresh¹ and The CHOICE Study.* 1) Johns Hopkins Medical Institutions, Baltimore, MD; 2) Laboratory of Genomic Diversity, NCI, Frederick, MD; 3) University of Vermont, Burlington, VT; 4) Basic Research Program, SAIC, NCI, Frederick, MD.

Hyperfibrinogenemia is common among dialysis patients and may be related to an elevated risk of cardiovascular disease. We tested the hypothesis that genetic variation in FGB, shown to explain 1-5% of fibrinogen level variation in the general population, plays an important role in elevated fibrinogen levels among dialysis patients. Methods: Plasma fibrinogen was measured on 678 dialysis patients a median of 3 months from the start of dialysis using an automated clot-rate assay. Seven polymorphisms of the beta-fibrinogen gene were determined. Haplotype analysis was conducted using the PHASE program to estimate haplotype frequency with stratification for race. Multiple linear regression was used to analyze the association of fibrinogen with the haplotypes assuming an additive model. Results: Mean [SD] fibrinogen concentrations were high and varied by ethnicity (Blacks: 357[120] mg/dl, Whites 382[120], $p=0.006$). Genotype frequencies were in Hardy-Weinberg equilibrium. Four common haplotypes identified were not associated with fibrinogen levels in the whole cohort, or after stratification by race. The A allele at 455, known to alter gene expression in vitro, was positively associated with fibrinogen levels only in non-diabetics (30 mg/dl for +1 copy of A allele, $p=0.034$, $R^2=2\%$). After adjustment for age, gender, race, smoking, baseline dialysis modality, comorbidity, and statin use, this association was only marginally significant (21 mg/dl for +1 copy of A allele, $p=0.07$). Conclusion: In this study, beta fibrinogen gene does not play an important role in determining variation in elevated plasma fibrinogen levels among dialysis patients. Funded in part by R01 L62985, AHA EI01-40197ND, & DHHS#NO1-CO-12400.

Susceptibility to Psoriatic Arthritis: influence of activating Killer Immunoglobulin-like Receptor genes. *M.P. Martin*¹, *G. Nelson*¹, *J-H. Lee*², *F. Pellett*³, *X. Gao*¹, *J. Wade*⁴, *M. Wilson*⁵, *J. Trowsdale*⁶, *M. Carrington*¹. 1) SAIC, NCI at Frederick, Frederick, MD; 2) LGD, NCI at Frederick, Frederick, MD; 3) Toronto Western Research Institute, and Centre for Prognosis Studies in the Rheumatic Diseases, Toronto Western Hospital, Toronto, Canada; 4) Regional HLA Laboratory, University Health Network, Toronto, Canada; 5) GlaxoSmithKline, Stevenage, UK; 6) University of Cambridge, Cambridge, UK.

Natural killer (NK) cell activity is controlled by a complex array of activating and inhibitory signals occurring in part through the interactions between killer immunoglobulin-like receptors (KIR) on the NK cell surface and their respective HLA class I ligands. The independent segregation of *HLA* and *KIR* genes along with specificity of each receptor for particular HLA allotypes raises the possibility that any given individual may express one or more KIR molecules for which no HLA ligand is present. Here we report that individuals who have genes encoding the activating *KIRs* *2DS1* and *2DS2*, are relatively susceptible to developing psoriatic arthritis, but only in the absence of HLA C ligands for the homologous inhibitory receptors *2DL1* and *2DL2/3* respectively. A model is presented in which absence of the ligands for inhibitory KIRs could potentially lower the threshold for NK (and/or T) cell activation mediated through activating receptors, thereby contributing to the pathogenesis of psoriatic arthritis. This project has been funded by DHHS #NO1-CO-12400, the Arthritis Society, and the Canadian Institutes for Health Research.

Interleukin-10 gene polymorphisms are associated with asthma phenotypes. *H. Lyon*^{1,3}, *C. Lange*², *E.K. Silverman*¹, *A. Randolph*^{1,3}, *D. Kwiatkowski*¹, *B.A. Raby*¹, *R. Lazarus*¹, *K. Weiland*¹, *N. Laird*², *S.T. Weiss*¹. 1) Channing Laboratory, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; 2) Harvard School of Public Health, Boston, MA; 3) Children's Hospital, Boston, MA.

Introduction: IL-10 is an anti-inflammatory cytokine reported to have lower protein levels in asthmatics. Since reduced IL-10 levels may influence the severity of asthma phenotypes, we examined IL-10 SNPs for association with asthma severity and allergy phenotypes.

Method: Utilizing DNA samples from 518 Caucasian asthmatic children from the Childhood Asthma Management Program (CAMP) and their parents, we genotyped six IL-10 SNPs: 3 in the promoter, 2 in introns and one in the 3' UTR. Using family-based association tests in the FBAT program, each SNP was tested for association with asthma and allergy phenotypes individually and by multilocus analysis. IgE, eosinophil counts, and number of positive antigen skin tests were used as allergy phenotypes. Asthma severity was assessed by measurements of post bronchodilator forced expiratory volume in one second (FEV1) percent predicted and response to methacholine as measured by log PC20. Bronchodilator response phenotypes were also assessed at baseline enrollment in the study. **Results:** One promoter SNP (-1119A>G) and the 3' UTR SNP (c4299T>C) were significantly associated with post bronchodilator FEV1 percent predicted ($p=0.025$ and 0.0002 , respectively). SNP c4299T>C was also associated with the number of positive skin tests ($p=0.003$). SNP -1119A>G was associated with IgE level ($p=0.049$). The multilocus analyses showed a significant association between asthma severity and all six SNPs ($p=0.006$) as well as the four SNPs that defined the most common haplotype in Caucasians ($p=0.005$). **Conclusions:** Polymorphisms in IL-10 are associated with asthma severity and allergy in this cohort. Further studies of variation in the IL-10 gene may help elucidate the mechanism of asthma development in children.

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Meta-analysis of the association of peroxisome proliferator-activated receptor (PPAR-gamma) *Pro12Ala* polymorphism with body mass index: a potential marker for diabetic tendency. *L.L. Roberts, J. Coresh, J.B. Hetmanski, T.H. Beaty.* Department of Epidemiology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD.

Peroxisome proliferator-activated receptor-g2 (PPAR-g2) is a nuclear receptor that regulates adipocyte differentiation and possibly insulin sensitivity with a common proline-to-alanine substitution (*Pro12Ala*). A number of association studies of this *Pro12Ala* variant with body mass index (BMI) have reached contradictory conclusions. We conducted a meta-analysis to examine rigorously whether the PPAR-g2 *Pro12Ala* variant is associated with higher BMI, document the extent of heterogeneity across studies, and assess whether measured covariates could explain this heterogeneity. A search of the literature revealed 33 subpopulations (17 Caucasian, 9 Asian and 7 Finnish) with relevant data published from 1998 to 2001. In the pooled sample size of 13,414 genotypes (2315 -/A variant, 11,099 P/P wildtype), the overall standardized mean BMI difference between variant -/A and wildtype P/P genotypes in a random effects meta-analysis was 0.11 kg/m² (95% CI [0.03, 0.18], p=0.004). However, heterogeneity of the association with BMI was highly significant ($c^2_{df=32} = 222.97$, $p < 0.0001$). The genotypic difference in mean BMI was significant for Caucasians (0.15 kg/m², 95% CI [0.04, 0.27]) and for the 18 published subpopulations without type 2 diabetes (0.19 kg/m², 95% CI [0.09, 0.29]), but the between study heterogeneity remains significant. In summary, the association of the PPAR-g2 *Pro12Ala* variant with BMI is weak overall and inconsistent across studies suggesting unidentified modifiers or publication bias without causal association.

Rapid Confirmation of Southeast Asian (--^{SEA}) and Filipino (--^{FIL}) α -Thalassemia Genotypes from Newborn Screening Samples. *U. Bhardwaj*¹, *Y.H. Zhang*¹, *L. McCabe*^{1,2}, *W. Blackburn*³, *E.R.B. McCabe*^{1,2,4}. 1) Department of Pediatrics, and; 2) Human Genetics, David Geffen School of Medicine at UCLA, Los Angeles, CA; 3) Hemoglobinopathy Screening Section, Texas Department of Health, Austin, TX; 4) Molecular Biology Institute, UCLA, Los Angeles, CA.

α -Thalassemia, the most prevalent genetic disorder, frequently results from the deletion of one (α^+ : $-a/$) or two (α^0 : $--/$) cis linked α -genes on chromosome 16. Homozygosity for α^0 -thalassemia ($--/--$) is a serious health problem and is responsible for the majority of hydrops fetalis in Southeast Asia. Asian immigration into the United States has impacted the demography of genetic diseases, particularly the hemoglobinopathies. Asians comprise 10% of California births and represent the population at highest risk for α^0 -thalassemia. The purpose of our investigation was to develop a single-tube PCR assay using neonatal dried blood specimens to identify individuals with α^0 -thalassemia due to the Southeast Asian deletion (--^{SEA}) and/or the Filipino deletion (--^{FIL}). A multiplex PCR assay was performed using the gap-PCR strategy. Primers were designed across the --^{SEA} and --^{FIL} deletion breakpoints. The PCR assay generated products for the normal genotype, as well as for the --^{SEA} and the --^{FIL} deletions. This technique was standardized on cell lines with known --^{SEA} and --^{FIL} deletions (NIGMS Human Mutant Cell Repository). We tested this multiplex PCR approach on 21 anonymized blood spots from neonates of Asian descent showing Hb Barts on newborn screening. We found that 12 samples were heterozygous for the --^{SEA} deletion and one for the --^{FIL} deletion. The other eight samples of the presumed α -globin defect may have had mutations other than --^{SEA} and --^{FIL}. We conclude that neonatal dried blood spots can be used to screen for severe α -thalassemia deletions among individuals of Southeast Asian and Philippine descent. We speculate that the implementation of DNA confirmation for α^0 -thalassemia using the original newborn screening sample will not only accelerate accurate diagnosis, but will also facilitate carrier detection, genetic counseling and prenatal diagnosis for future pregnancies in these families.

Investigation of the age dependent genotype frequencies of candidate genes for coronary atherosclerosis in healthy blood donors. A. Kabisch¹, S. Schulz², P. Greiser², U. Mueller-Werdan³, K. Werdan³, C. Glaeser². 1) Bloodbank, MLU, Halle, Germany; 2) Inst. of Human Gen., MLU, Halle, Germany; 3) Dep. of Intern. Med., MLU, Halle, Germany.

Atherosclerosis is a complex disease influenced by a variety of environmental and genetic factors. Among the genetic factors there are a lot of well investigated established risk markers like e.g. polymorphisms (PM) in the ACE-, ApoE-, and E-selectin-gene as well as the Leiden-PM in the factor V-gene. Methods: We investigated the genotype distribution of the above-mentioned PM in a group of 362 long-standing healthy blood donors without any coronary symptoms and coronary risk factors including hypertension, smoking, diabetes mellitus and drug treatment (medical monitoring >3y; mean age: 41.9y; 61.3% male). All blood donors belong to the same Central German Caucasian origin. Results: For evaluation of the genotype distributions the DD- (ACE), the 3/4- (ApoE), the Arg/Arg- (E-selectin) as well as the AA-genotype (factor V) were considered pathologically in terms of coronary atherosclerosis in common consent. We evaluated the score of the pathological genotypes taking the gender as well as the age (18-29y vs. 30-39y vs. 40-49y vs. 50-70y) into consideration. We could not detect any gender specific significant differences in the score within the group of healthy blood donors irrespective of age ($p=0.542$) even though it is known that there is a gender specific preference to the incidence of coronary afflictions. In addition one could imagine that with increasing age the number of these pathological genetic risk markers should be decreased considerably in the group of healthy blood donors because of the supposed cardiovascular susceptibility of persons carrying these genotypes. Surprisingly this expected decrease with age was not found among the healthy blood donors ($p=0.337$). Conclusions: Our results suggest that the investigated genetic markers are not predictors for the incidence of coronary atherosclerosis in general. These findings do not rule out that these PMs could be markers for the progress and therefore for a practicable clinical therapy of the complex atherosclerotic disease.

Genetic Risk Factors for Pediatric Tuberculosis Disease. *S. Malik*^{1,2}, *L. Abel*³, *H. Tooker*⁴, *A. Poon*¹, *L. Simkin*¹, *M. Girard*¹, *G. Adams*⁴, *J.R. Starke*⁴, *K. Connely-Smith*⁴, *E.A. Graviss*⁴, *J.M. Musser*⁵, *E. Schurr*^{1,2,6}. 1) McGill Centre for the Study of Host Resistance; 2) Department of Medicine, McGill University, Montreal, PQ, Canada; 3) INSERM U550, Hopital Necker, Paris, France; 4) Baylor College of Medicine, Houston, TX; 5) Laboratory of Human Bacterial Pathogenesis, NIH, Hamilton, MT; 6) Department of Human Genetics, McGill University.

Tuberculosis, an infectious disease caused by *Mycobacterium tuberculosis* kills an estimated 2 million people per year. There is strong evidence that tuberculosis susceptibility is strongly influenced by genetic factors. We undertook a family based association study between genetic markers in selected candidate genes and pediatric ("primary") tuberculosis. A total of 183 nuclear families with pediatric tuberculosis cases were enrolled from Harris County, Texas where the incidence of pediatric tuberculosis is 3.1/100 000, well above the US national average of 1.6/100 000. The majority of families, 143, comprised only a single tuberculosis case (simplex families) while in 41 families more than one case was diagnosed (multiplex families). With regard to ethnicity and racial background of tuberculosis patients, 136 were of Latino origins, 69 were Blacks, 13 Asian, 7 White, and 9 of mixed racial origin. The disease manifestation was classified as pulmonary in 57.3%, extrapulmonary in 37.5%, and mixed pulmonary and extrapulmonary in 11.2% of all pediatric cases. Finally, 100 pediatric cases were female and 106 were male. Candidate genes (*NRAMP1*, *MBL*, *VDR*) were chosen based on previous genetic findings and immunopathogenic studies of tuberculosis. Alleles of all candidate genes tested were in Hardy-Weinberg equilibrium. Associations between disease phenotype and three allelic variants, *NRAMP1*-N01 ($P= 0.04$), *NRAMP1*-N02 ($P= 0.04$), and *VDR*-VD01 ($P= 0.02$) were found significantly associated with pediatric tuberculosis. The effect of *NRAMP1*-N02 allele transmission distortion was significantly more evident in simplex families ($P= 0.0005$).

Meta-analysis of genetic association studies supports a role for common variants in common disease risk. *J.N. Hirschhorn*^{1,2,3}, *K.E. Lohmueller*³, *C.L. Pearce*⁴, *M. Pike*⁴, *E.S. Lander*^{3,5}. 1) Genetics, Harvard Medical School, Boston, MA; 2) Genetics and Endocrinology, Children's Hospital, Boston, MA; 3) Center for Genome Research, Whitehead Institute, Boston, MA; 4) Norris Comprehensive Cancer Center, USC, Los Angeles, CA; 5) Biology, MIT, Cambridge, MA.

Association studies offer a potentially powerful approach to identify common genetic variants that influence susceptibility to common disease, but are plagued by the impression that they are not consistently reproducible. In principle, the inconsistency may be due to false positive studies, false negative studies, and/or true variability in association between different populations. The critical question is whether false positives overwhelmingly explain the inconsistency.

We analyzed 301 published studies covering 25 different reported associations. There was a large excess of studies replicating the first positive reports, inconsistent with the hypothesis of no true positive associations ($P < 10^{-14}$). This excess of replications could not be reasonably explained by publication bias, and was concentrated among 11 of the 25 associations. For 8 of these 11 associations, a pooled analysis of follow-up studies yielded a statistically significant replication of the first report, with modest estimated genetic effects that would be difficult to detect using small samples. Thus, a sizable fraction (but well under half) of reported associations have strong evidence of replication; for these, false negative, underpowered studies probably contribute to inconsistent replication. We conclude that there are likely many common variants in the human genome with modest but real effects on common disease risk, and that studies utilizing large samples will convincingly identify such variants.

Prediction of hypertensive status by MDR analysis of multilocus genotypes. *S.M. Williams^{1,2,3}, M.D. Ritchie³, J.A. Phillips III^{2,4}, E. Dawson⁵, M. Prince⁴, E. Dzshura⁴, A. Willis^{3,4}, A. Semanya¹, M. Summar^{3,4}, J.H. Addy⁶, J. Kpodonu⁶, L.-J. Wong⁷, R.A. Felder⁸, P.A. Jose⁹, J.H. Moore³*. 1) Dept Micro, Meharry Med Col, Nashville, TN; 2) GCRC, Vanderbilt Univ Med School, Nashville, TN; 3) Prog in Hum Gen, Vanderbilt U Med School, Nashville, TN; 4) Div of Med Gen, Dept of Ped, Vanderbilt U School of Medicine, Nashville, TN; 5) Bioventures, Murfreesboro, TN; 6) Dept of Med, U of Ghana Med School, Accra, Ghana; 7) Inst for Hum and Mol Gen, Georgetown U Med Ctr, Washington, DC; 8) Dept of Path, U of VA Health Sci Ctr, Charlottesville, VA; 9) Dept of Ped and Phys and Biophys, Georgetown U Med Ctr, Washington, DC.

While hypertension is recognized as a complex disease with a significant genetic component, most genetic studies of hypertension fail to identify variants that can be replicated across populations. We hypothesize that this is due to differences in underlying genetic architecture that is rarely investigated. We previously demonstrated that multilocus interactions are risk factors for hypertension. To determine the specific multilocus genotypes that underlie hypertension we used a new methodology, multifactor-dimensionality reduction (MDR) analysis, to identify high-risk and low-risk genotypes. We analyzed 13 polymorphisms in 8 genes (angiotensinogen - AGT, angiotensin-I-converting enzyme - ACE, G protein-coupled receptor kinase GRK4, nitric oxide synthase I - NOS1, NOS3, carbamyl phosphate synthase I - CPSI and cyp2C8). The genes play a role in 4 physiological pathways. None of the polymorphisms associate with hypertension individually, but genotypes at four polymorphisms in three unlinked loci, AGT, ACE, and GRK4 predict phenotype correctly in approximately 70% of the individuals in our study. These genes play a role in vasoconstriction (AGT, ACE) or in sodium excretion and fluid volume (GRK4). Our findings support the idea that combinations of genotypes from multiple loci provide more information about hypertension susceptibility than genotypes from any single gene, and that the role of specific candidate genes is dependent on variation at some, but not all, other loci with a proposed role in hypertension.

Association of a novel promoter variation in the peroxisome proliferator-activated receptor-g coactivator-1 (PGC-1/PPARGC1) gene with body mass index (BMI). *T.I. Pollin¹, Z. Fan², M.J. Garant¹, D.G. Lewis¹, B.M. Spiegelman³, B.D. Mitchell¹, E.T. Poehlman⁴, A.R. Shuldiner¹.* 1) University of Maryland, Baltimore, MD; 2) University of Missouri, Columbia, MO; 3) Harvard University, Boston, MA; 4) University of Montreal, Montreal, Canada.

We previously reported association of homozygosity (AA) for a polymorphism in intron-2 (C®A) of the PGC-1 gene with protection against obesity in Caucasians. We also found evidence for interaction of this variant with the b-3 adrenergic receptor (b3AR) Trp64Arg variant, consistent with functional studies showing that stimulation of the b3AR induces PGC-1 expression. We now report the identification and analysis of a novel 8 base pair expansion of a 13 poly-A segment in the promoter region of PGC-1. This variant was genotyped in 829 women and 66 men and was in partial linkage disequilibrium with the intron-2 variant ($D' = 0.46$, $p < 0.0001$). The promoter variant was not by itself associated with BMI, but inspection of the BMI levels for each of the nine possible promoter/intron-2 genotype combinations pointed to a recessive model in which individuals homozygous for the 13A/intron-2A haplotype were leaner than those with other genotypes (age- and sex- adjusted BMI: 29.3 ± 1.0 vs. 32.8 ± 0.3 kg/m², $p = 0.0007$).

Our previous detection of a combined PGC-1/b3AR association with BMI was reinforced by the difference in BMI between those with 13A/intron-2A homozygosity and the b3AR Trp/Trp genotype (28.8 ± 1.1 kg/m²) and those with other PGC-1/b3AR genotype combinations (32.8 ± 0.3 kg/m²) ($p = 0.0005$).

In summary, we observed an association between a novel 8 base pair insertion in the promoter of the PGC-1 gene and BMI when this variant was combined with a single nucleotide polymorphism in the second intron, and a PGC-1/b3AR interaction was supported. These results may reflect a functional relationship whereby the combination of the two PGC-1 variants alters expression of PGC-1 protein, which in turn augments the known functional effect of the Trp64Arg b3AR variant on adrenergic signaling, lipolysis, and fat accretion.

Use of Regression Trees to Detect Linkage of Rheumatoid Arthritis to Chromosome 1. *C.J. Etzel¹, D. Jawaheer², M.F. Seldin³, W.V. Chen¹, P.J. Gregersen², C.I. Amos¹.* 1) Dept of Epidemiology, The University of Texas MD Anderson Cancer Center, Houston, TX; 2) Division of Biology and Human Genetics, North Shore University Hospital, Manhasset, NY; 3) Dept of Biological Chemistry, University of California at Davis, Davis, CA.

Rheumatoid arthritis (RA) is a debilitating chronic, inflammatory disease that affects the synovial membranes of multiple joints in the body. The etiology of the disease remains unknown, but appears to have a complex genetic component. The North American Rheumatoid Arthritis Consortium (NARAC) recently completed a genome scan of multiplex RA families from within the United States. We found significant evidence ($p < 0.01$) for linkages in two separate sets of families to two regions on chromosome 1. Subsequent affected relative pair linkage analysis on all the NARAC sib-pairs allowing for covariates using LODPAL at the marker H18a (136.9 cM) yielded a LOD score of 1.53 including erosive disease and 1.09 without covariates. We constructed classification and regression trees to search for subsets of the data providing the strongest evidence for linkage to this locus on chromosome 1 using 523 affected sib-pairs from NARAC. Our response was the proportion of alleles shared IBD for H18a and we considered the following predictors: ethnicity and numbers of sibs per sib-pair with: one or more DR4 alleles, erosive factor, rheumatoid factor > 100 (RF), male affected, and existence of nodules. We compared the results from the tree analyses to the linkage results. From LODPAL, the covariates male affected, RF, DR4 and erosive factor nonsignificantly increase evidence for linkage to this region of chromosome 1. The results from classification and regression tree analysis identified a subgroup of sib pairs that shared a very high proportion of alleles i.b.d. for H18a. For these pairs, both members had erosive factor, at least one member had a nodule, both sibs were female and at least one pair member had RF > 100 . These results indicate a subgroup that is particularly useful for further fine mapping studies.

Segregation Analysis of Total Serum IgE in a Completely Ascertained Isolated Island Population. *R.A. Mathias¹, T.H. Beaty¹, J. Bailey-Wilson², A. Wilson², K.C. Barnes¹*. 1) Johns Hopkins Univ, Baltimore, MD; 2) NHGRI, NIH, Baltimore, MD.

Total serum Immunoglobulin E (tIgE) is a quantitative trait highly correlated with asthma and plays a major role in its pathophysiology. We performed a segregation analysis of tIgE levels in a genetic isolate under a random ascertainment scheme. Genealogical data dating back to 1722 and spanning 13 generations for 664 individuals living in Tangier Island, Virginia, were available. All individuals belonged to one extended pedigree (N=3,501). Pedigree complexity indicates that current inbreeding ($F = 0.009$) is only due to old lines of consanguinity. Serum samples for tIgE concentrations were collected from 445 (68.2%) of the population. Computation time needed for the segregation analysis using Pedigree Analysis Package was evaluated for several alternate pedigree structures. Only two pedigree reconstructions were feasible: a *reduced* pedigree retaining all phenotyped individuals and their parents (57 pedigrees), and the breakup into *nuclear* components with the duplication of individuals as necessary (922 pedigrees). Age- and gender-adjusted log-transformed tIgE was used. Both the *reduced* and *nuclear* pedigrees indicated that the most parsimonious model for log(tIgE) in these data is a two-distribution Mendelian model. There was ambiguity between dominant and recessive models, and this putative major gene accounted for all the familial correlation in the trait. However, codominant and non-genetic models could not be rejected. Simulations of a quantitative trait were performed to evaluate the reliability of this most parsimonious model. Simulations over 200 replicates for both pedigree structures indicated the results obtained when using the restricted genealogical information for the two-distribution Mendelian model were reliable. In conclusion, it appears that tIgE may have a strong genetic component in this Tangier Island population with two underlying distributions, and a majority of founders falling into the lower phenotypic distribution. Additionally, the results based on either the reduced and nuclear pedigrees appear to be quite reliable for the most parsimonious model.

An SNP Map for Admixture Linkage Disequilibrium Studies in African Americans. *J.A. Lautenberger¹, J.-P. Chretien^{1,2}, J. Coresh², S.J. O'Brien¹, M.W. Smith³*. 1) Laboratory of Genomic Diversity, National Cancer Institute, Frederick, MD; 2) Welch Center for Prevention, Epidemiology, and Clinical Research, Johns Hopkins University, Baltimore, MD; 3) Basic Research Program, SAIC-Frederick, National Cancer Institute at Frederick, Frederick, MD.

Association mapping requires linkage disequilibrium (LD) between a disease susceptibility locus and a marker. In non-admixed human populations, significant LD is limited to several hundred Kb which is equivalent to a few tenths of a cM. A novel method of gene mapping, mapping by admixture linkage disequilibrium (MALD), has been developed to take advantage of the increased LD across 5-25 cM in admixed populations. Many fewer highly differentiating markers must be typed using a MALD screen as compared to the proposed haplotype approach in a non-admixed population. We focus on African Americans (20% average European ancestry) as a suitable population for MALD mapping of disease genes.

We have analyzed a set of 27,054 single nucleotide polymorphisms (SNPs) from the SNP Consortium allele frequency database. Markers having at least 20 subjects typed in both African and European Americans were examined with Var(MAPP*) - a measure of affiliation with parental population that takes centrality and directionality of allele frequencies into account. 61% of the human genome is within 2.5 Mb of an SNP with Var(MAPP*) > 6 and 89% is within 7.5 Mb. Under reasonable assumptions, about 1,200 cases and an equal number of controls would be required to detect an association between a disease locus with a genotypic risk ratio of two or greater and such a marker located within 2.5 cM. After ongoing verification, these informative SNPs constitute the foundation of a suitable set of markers for genomic MALD scans in African Americans. Funded in part by DHHS#NO1-CO-12400.

Association between Viliuisk Encephalomyelitis syndrome in Sakha People of Siberia and the inflammation gene polymorphisms. *T.K. Oleksyk¹, T. Sivtseva², A.P. Danilova², V.L. Osakovskiy², S.J. O'Brien¹, L.G. Goldfarb³, M.W. Smith⁴.* 1) Laboratory of Genomic Diversity, National Cancer Institute, NIH, Frederick, MD; 2) Institute of Health, Sakha Republic, Russia; 3) Clinical Neurogenetics Unit, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD; 4) Basic Research Program, SAIC Frederick, National Cancer Institute, NIH, Frederick, MD.

Since the discovery of Viliuisk Encephalomyelitis (VE) in 1887 scientists have tried to understand the etiology and identify the causative agent of this endemic neurological disorder common among the native Sakha population of Central Siberia. Analysis of VE occurrence in affected families indicated a greater than expected by chance aggregation of VE in relatives, suggesting that a genetic component may contribute to VE susceptibility. Our main objective was to analyze the degree of association between VE and genetic markers in several candidate genes that have been implicated in other inflammatory diseases. DNA samples were collected from 83 affected families comprising 89 verified VE cases. DNA of additional 72 unrelated unaffected inhabitants of the same geographical area was obtained. We tested seven inflammation candidate genes: CCR2/5, IFNG, IL4, IL10, MBL, SDF, and RANTES using 24 variable SNPs to perform fluorescent ddNTP length-modified single base extension. Results were analyzed using the Transmission/Disequilibrium Test (TDT) by inferring the genotypes of missing parents. The degree of association between the SNP markers and VE in affected versus the unrelated reference individuals using inferred haplotypes was also examined. None of the seven genes were significantly associated with VE occurrence. However, results of the TDT tests with the SNP markers located in the IL10 region were marginal ($p \sim 0.07$) which suggests that further work around this gene could be fruitful. This collection of individuals and the analytic approach has laid the foundation for discovering the natural human variants may contribute to the identification of the etiology of Viliuisk Encephalomyelitis. Funded in part by DHHS # NO1-CO-12400.

Population subisolate structure approach for complex gene mapping. *J.K. Kere*^{1,2}, *E. Salmela*², *P. Lahermo*², *P. Sistonen*³, *M.-L. Savontaus*⁴. 1) Dept Biosciences at Novum, Karolinska Inst, Huddinge, Sweden; 2) Finnish Genome Center, Univ Helsinki, Finland; 3) FRC Blood Transfusion Service, Helsinki; 4) Dept Med Genet, Univ Turku, Finland.

Finland is a model population for disease gene studies. Much debate has focused on the utility of founder populations for disease gene mapping, and it has been suggested that such populations offer no advantages. In Finland, linkage disequilibrium (LD) levels are similar to other European populations for common alleles. Such data are compatible with a relatively broad (hundreds of founders) bottleneck, whereas examples of rare alleles (<1%) have shown long segments of LD. We aim at obtaining a more comprehensive picture of the substructure of the Finnish population, to aid the design of genetic studies of complex diseases. Distinct substructuring was already suggested by classical blood group studies as well as the uneven geographical spread of rare disease alleles, and was confirmed by our microsatellite studies on >450 individuals from 9 provinces. Allele frequencies for 31 markers deviated by up to 3.5 SD around the national mean in different provinces, suggesting local drift. Principal component (PC) analysis revealed that the 1st and 2nd PCs recovered 47% of total variance, and the geographical interpretation of the 1st PC was along an east-west axis. We have selected additional markers to study LD in different provinces. We propose a novel strategy for the mapping of common disease genes. The steps of the strategy are the following: 1. Look for subpopulations with very different incidence for a common disease, 2. Collect representative population samples, 3. Scan the genome for markers and regions that have very different allele distributions between the subpopulations (most of the allele frequency variations are due to drift, but some may mark disease-associated haplotypes), 4. Perform an association study with that subset of markers between cases and controls to find true disease association, and 5. Replicate the mapping result in independent populations. We conclude that a distinct substructuring verified in Finland may allow the use of novel strategies for mapping common disease genes.

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Linkage Disequilibrium across 150 kb region around RET gene on chromosome 10. *N. Mukherjee, A.J. Pakstis, P. Chaudhuri, W. Speed, J.R. Kidd, K.K. Kidd.* Dept.Genetics, Yale U, New Haven, CT.

Because recombination can be a significant factor in reducing LD, regions of low recombination rates per Mb should have LD extending for longer distances than regions of known high recombination rates. One region of known reduced recombination rate relative to the genome average is the centromeric region of 10q around the RET locus. To examine LD in this region we have identified SNPs at approximately 30kb interval across ~300 kb from RET to D10S94 and are typing these markers in over 30 populations from major continental regions. Preliminary analysis of the several markers with sufficiently high heterozygosities demonstrate high levels of pair-wise LD extending across segments of >150kb in this region but with considerable variation among populations. For a 100kb region extending telomeric from RET, most populations in all regions outside of Africa showed strong and highly significant LD with D' generally greater than 0.6 and $p < 0.001$. LD was difficult to assess for many segments in African populations because of low heterozygosity for one or both markers. A permutation test of LD across a segment of 137 kb using 3-site haplotypes at each end shows highly significant LD in most populations, even in some African populations. However, some individual populations showed no suggestion of any LD. We conclude that for most populations in most regions of the world, LD does extend farther in this centromeric region of reduced recombination than seen for the average mid-arm genomic region. Supported in part by GM 57672.

Strong linkage disequilibrium for the frequent 35delG GJB2 mutation in the Greek population. *S. Pomoni*¹, *L. Van Laer*², *M. Grigoriadou*¹, *A. Pampanos*¹, *T. Iliades*³, *N. Voyiatzis*³, *J. Economides*⁴, *P. Leotsakos*⁴, *P. Neou*⁵, *M. Tsakanikos*⁵, *A. Skevas*⁶, *G. Van Camp*², *M.B. Petersen*¹. 1) Inst. of Child Health, Athens, Greece; 2) Univ. of Antwerp, Belgium; 3) Aristotle Univ. of Thessaloniki, Greece; 4) "Aghia Sophia" Children's Hosp., Athens, Greece; 5) "P. & A. Kyriakou" Children's Hosp., Athens, Greece; 6) Univ. of Ioannina, Greece.

Mutations in the GJB2 gene represent the most important cause of nonsyndromic, prelingual deafness. One specific mutation in this gene, the 35delG, has accounted for the majority of GJB2 mutations detected in Caucasian populations and represents one of the most frequent disease mutations identified so far. High carrier frequencies of the 35delG mutation have been detected in southern European countries. Most investigators have assumed that the high frequency of the 35delG mutation reflects the presence of a mutational hot spot, but a recent study including patients from Belgium, U.K., and the U.S.A. reported evidence of a founder effect. In the present study we genotyped 60 Greek deafness patients homozygous for the 35delG mutation for 6 SNPs and one microsatellite marker, mapping within the 3'UTR or flanking the GJB2 gene. The allele distribution in the patients was compared to 60 Greek hearing controls. On the centromeric side of the GJB2 gene, no significant difference in genotypes was detected between patients and controls using SNP5 and SNP6 located at a distance of 76.5 and 62.8 kb from the 35delG mutation, respectively. The two SNPs within the 3'UTR showed a strong association with the 35delG mutation ($p < 0.001$ for SNP1 and $p = 0.013$ for SNP2). On the telomeric side of GJB2, two SNPs showed significant genotype differences between patients and controls (SNP3 at 5.5 kb distance, $p = 0.004$ and SNP4 at 6.0 kb distance, $p < 0.001$). Surprisingly, the microsatellite marker D13S175 located at a distance of about 90 kb telomeric to GJB2 showed a strong association between the 105 bp allele in patients (92.9%) compared to controls (47.3%, $p < 0.0005$). We conclude that the 35delG mutation is in strong linkage disequilibrium with intragenic and flanking markers in the Greek population supporting that this mutation is derived from a common founder.

Positive results in association studies are associated with departure from Hardy-Weinberg Equilibrium: hint for genotyping error? *A.R. Turner, J.L. Little, E.R. Bleecker, D.A. Meyers, J. Xu.* Center for Human Genomics, Wake Forest Univ Sch Medicine, Winston-Salem, NC.

Case-control studies have been widely used in association studies of complex diseases. The premise of genetic association studies is that increased allele or genotype frequencies in cases compared with controls implicates sequence variants that increase risk to a disease, or are in strong linkage disequilibrium (LD) with a disease-causal mutation. However, many other factors can also lead to an observed difference in allele or genotype frequencies between cases and controls. While much attention has been devoted to the potential impact of incomparability between cases and controls in terms of sources of cases and controls, environmental exposures, and genetic background (population stratification), there is a clear lack of comprehension of the impact of genotyping error on the results of association studies. This may be due to the lack of direct measures of this type of error. To indirectly assess the prevalence and magnitude of genotyping error in case-control studies, we systematically reviewed reported association studies from PUBMED and performed Hardy-Weinberg Equilibrium (HWE) tests in control subjects for each reported single nucleotide polymorphism (SNP), as deviation from HWE may indicate genotyping error. We searched for keywords "association genotype genetic case control", and limited to articles in English, with human subjects, and 2000 publication date. The search yielded 157 articles, which we then limited to the 101 available articles. Our analysis revealed significant deviation from HWE among 12» (n=16) of the SNP genotypes ($\chi^2=4.22$, $P=0.04$). Also, we found more deviation from HWE among positive association studies (18.9»; n=10). We offer several suggestions to reduce systematic biases: 1) Blind researchers to case-control status, 2) Include multiple controls and blanks in different positions of each plate, 3) Check allele scoring, 4) Run one plate in duplicate for each gene, 5) Determine an acceptable amount of missing data and rerun assays if there is more missing data, 6) Perform a HWE test for each SNP before testing hypotheses.

Genetic evidence for “The Trail of Tears”: signatures of expansion preceded by a bottleneck in the Choctaw population of Oklahoma. *X. Zhou*¹, *N. Wang*², *F.K. Tan*¹, *M.F. Foster*³, *F.C. Arnett*¹, *R. Chakraborty*². 1) Division of Rheumatology and Clinical Immunogenetics, University of Texas-Houston Medical School, Houston, TX; 2) Center for Genome Information, University of Cincinnati, Cincinnati, OH; 3) Department of Anthropology, University of Oklahoma, Norman, OK.

Previous research has shown that the Choctaw Indians of Oklahoma exhibited considerable linkage disequilibria (LD) in a number of regions of the genome that has allowed genetic fine mapping for potential susceptibility genes for the autoimmune connective tissue disease scleroderma or systemic sclerosis (SSc). In principle, such enhanced background LD in the Choctaw could be caused by the recent expansion of their population size preceded by a population bottleneck. This investigation utilized partial genome-scan data on 54 dinucleotide loci from 76 Choctaw individuals that were used as controls in our previous SSc linkage studies, to seek genetic evidence of the known demographic history of the Choctaw Nation often referred to as The Trail of Tears. According to historical records, the Choctaw Nation was relocated to Oklahoma, from their original homeland in Mississippi in 1830-1850 during which time only 7,000 out of over 20,000 survived. Recent estimates of their current population size are approximately 70,000 suggesting a 10-fold increase in about 8 to 10 generations. The average expected homozygosity for the Choctaws at the 54 di-nucleotide loci (36.2%) was significantly higher than that in European-Americans (24.8%), and when adjusted for sample size differences, the Choctaws also exhibited a smaller number of segregating alleles at these loci. Both these observations are consistent with the trend expected in an isolated population. Comparison of the allele size variance and gene diversity yielded an imbalance index of 1.19 in the Choctaw. Of the 54 loci examined, 53 exhibited an excess of expected homozygosity in comparison to the expectations of a stepwise mutation model in a population of constant size. Taken together, these observations are consistent with a signature of the recent population size expansion of the Choctaws, preceded by a bottleneck event that occurred 8 to 10 generations ago.

Population genetics of the angiotensin-1 converting enzyme (ACE) locus in global populations. *M. Wolujewicz¹, R. Kaushal¹, N. Wang¹, G. Sun¹, E. Lutkenhoff¹, S.T. McGarvey², L. Jin¹, R. Chakraborty¹, R. Deko¹.* 1) Center for Genome Information, Department of Environmental Health, University of Cincinnati, Cincinnati, OH; 2) International Health Institute and Department of Community Health, Brown University, Providence, RI.

We have typed the *Alu* indel (I/D) polymorphism in intron 16 of the *ACE* gene, previously thought to be associated with plasma ACE activity, in 1262 individuals belonging to 26 worldwide populations. We observed a distinct cline of the *Alu**I allele increasing geographically from Africa (.34), extending through Europe (.40), South Asia (.54) and East Asia (.72), with the highest frequency in Oceania (.85). A sample (N = 39) of chimpanzees was surprisingly found to be polymorphic (*I = 0.18), contradicting the earlier assertion that the *ACE-Alu* indel locus is human specific. Sequencing establishes that this polymorphism predates the human-chimpanzee split. To further characterize the population genetic properties of the *ACE* gene, we have analyzed three additional SNPs downstream to the *Alu* locus in Benin from Africa (N = 56), German (N = 59), Chinese (N = 62), Samoan (N = 90), and the chimps (N = 38). One of the SNPs, 22982, earlier shown to be in strong linkage disequilibrium (LD) with ACE determining variants, is in complete LD (D' = 1) with the *Alu* indel locus among the Samoans, an isolated Polynesian population. These two sites are approximately 9 kb apart. A geographic cline of the "IA" haplotype (*Alu**I/22982*A) similar to the *Alu**I is evident in these populations. The two other SNPs (17634 and 20120), located between the above two markers, are monomorphic in the examined populations other than the Benin and the Samoan. At the 4-locus haplotype level, of the possible eight haplotypes, six are found in the combined sample of 305 individuals. Haplotype diversity is the highest in Africa, lowest in Samoa (lower than the chimpanzees) and intermediate in Europe and Asia. Together with the least number of observed haplotypes (4), the Samoans present the strongest evidence of an LD block, which has important implications for disease-gene association mapping in isolated human populations.

GABA_A receptor genes on chromosome 15 associated with alcohol dependence in the Collaborative Study on the Genetics of Alcoholism sample. *D.M. Dick¹, X. Xuei¹, J. Song¹, H.J. Edenberg¹, T. Foroud¹, COGA*

collaborators^{1,2,3,4,5,6,7,8}. 1) Indiana Univ Sch Medicine, Indianapolis, IN; 2) University of Iowa; 3) University of California at San Diego; 4) University of Connecticut; 5) State University of New York, Health Sciences Center at Brooklyn; 6) Washington University in St. Louis; 7) Rutgers University; 8) 8.

Several lines of evidence suggest that GABA is involved in many of the behavioral effects of alcohol, including motor incoordination, anxiolysis, sedation, withdrawal, and ethanol preference. GABA_A receptor agonists tend to potentiate the behavioral effects of alcohol, while GABA_A receptor antagonists attenuate these effects. Chromosome 15 contains a cluster of GABA_A receptor genes: *GABRA5*, *GABRB3*, and *GABRG3*. *GABRA5* and *GABRB3* were reported to be uniparentally expressed from the paternal chromosome, and an earlier analysis of microsatellite markers in those genes showed evidence for association with the paternally transmitted alleles. To follow up this finding and narrow the region, seven SNPs were genotyped in more than 2200 individuals from 262 multiplex alcoholic families ascertained as part of the Collaborative Study on the Genetics of Alcoholism (COGA). Both pedigree disequilibrium tests (PDT) and the classic trio based TDT tests were performed, and the TDTEX program in S.A.G.E. was used to test for paternal transmission. Using the TDT programs there was consistent evidence of association with a SNP in *GABRG3* with three hierarchical models of alcohol dependence. This association was due to excess paternal transmission. There was also some evidence of association with a SNP in *GABRB3* using the PDT. These results suggest that further investigation of the chromosome 15 GABA gene cluster is warranted to elucidate the role of these genes in the development of alcohol dependence. We are currently in the process of testing additional SNPs in this region.

Accounting for heterogeneity in a power analysis exemplified by ovarian ageing. *K.M. Asselt van*^{1,2,3}, *H.S. Kok*^{1,2,3}, *P.L. Pearson*³, *E.R. te Velde*², *D.E. Grobbee*¹, *L.A. Sandkuijt*⁴. 1) Julius Center, UMCU, NL; 2) Infertility Studies, UMCU, NL; 3) Medical Genetics, UMCU, NL; 4) Medical Statistics, LUMC, NL.

Objectives: Age at menopause is under influence of various genes and has been estimated to have a heritability of 60-85% in several studies. The range of normal menopausal age is between 40-60 years with a mean of 50-51 years. ~1% of all women cease menstruating prior to the arbitrarily selected cut-off of 40 years and exhibit Premature Ovarian Failure (POF). Various genetic studies suggest that POF has a different etiology to natural menopause and is caused by single chromosomal or gene mutations. Segregation analysis show that both women with POF as women with early menopause (40-45 years) co-segregate in families suggesting that the natural menopause distribution overlaps with the POF distribution. To successfully perform a genome scan to search for genes responsible for the variation in age of normal menopause, we need to take account of this heterogeneity. **Methods:** We collected sisters with concordant (both <46 or both >54) and discordant ages at menopause. In the lower tail of the distribution a mixture of women with monogenetic POF causes and women with a normal early menopause is possibly collected. To estimate the power of the sib pair analysis we have used a procedure in which both concordant pairs from the upper tail of the distribution and discordant pairs were used. By doing so we hope to minimize mapping putative strong POF genes which might have occurred if pairs from the lower end of the distribution were used. The IBD probabilities were calculated with the method of Risch and Zhang using the known menopausal ages, the mean menopausal age and the corresponding standard deviation of a population based cohort. These IBD probabilities were used with simulated marker data in a Haseman-Elston model. **Results:** The power to find at least one locus in the presence of 6 responsible genes with a heritability of 80% and with allele frequencies of 0.1 and 0.25 varied between 85-99% (p<0.01). **Conclusions:** The dataset (158 sib-pairs) and sib-pair selection procedure appears adequate to perform a linkage study in a genome screen for variation in age at menopause.

Linkage of the *APOE* locus with ApoE plasma levels in adolescent Dutch twins is completely explained by the *APOE* e2/e3/e4 alleles. M. Beekman¹, D. Posthuma², BT. Heijmans¹, N. Lakenberg¹, HED. Suchiman¹, C. Klufft³, GP. Vogler⁴, P. de Knijff⁵, GJB. van Ommen⁵, RR. Frants⁵, PE. Slagboom¹, DI. Boomsma². 1) Section Molecular Epidemiology, LUMC, Leiden, Netherlands; 2) Dept of Biological Psychology, Free University of Amsterdam, Netherlands; 3) Dept of Vascular and Connective Tissue Research, TNO Prevention and Health, Leiden, Netherlands; 4) Center for Developmental and Health Genetics and Dept of Biobehavioral Health, Pennsylvania State University, USA; 5) Dept of Human and Clinical Genetics, LUMC, Leiden, Netherlands.

ApoE plasma level and isoform influence the clearance rate of lipoprotein particles by the liver. *APOE* e2/e3/e4 alleles underlying the isoforms have been indicated to be one of the determinants of ApoE plasma level. The effect of additional genetic variation at the *APOE* locus remains unclear. We determined the contribution of *APOE* e2/e3/e4 alleles to variation in ApoE plasma level and tested for the existence of additional genetic variation within the *APOE* region influencing ApoE levels using a recently developed combined linkage-association approach. We genotyped the *APOE* e2/e3/e4 polymorphism and 16 short tandem repeats on chromosome 19 in 65 monozygotic and 82 dizygotic Dutch twins. A two-step analysis procedure was applied. First, multi-point variance-components linkage analysis was performed. A putative QTL explaining 38% of the variation in ApoE plasma level was found at the *APOE* locus (19q13.2; p=0.08). Next, linkage and association with the *APOE* e2/e3/e4 alleles were modelled simultaneously. Association was implemented as the between and within twin pair effect of the alleles, allowing to separate genuine associations from associations due to population stratification. In the combined analysis, association with e2/e3/e4 alleles explained 41% of the variation in ApoE level (p<0.0005) and neutralized the linkage due to the putative QTL. Thus, the complete linkage of the *APOE* locus with ApoE level can be attributed to the e2/e3/e4 alleles. These findings exclude the presence of other genetic variation at the *APOE* locus with a major independent effect on ApoE plasma level in adolescent Dutch twins.

The segregation distortion of MTHFR haplotypes is not increased in DZ twinning. *G.W. Montgomery¹, D.L. Duffy¹, K.I. Morley¹, Z.Z. Zhao¹, A.J. Marsh¹, D.I. Boomsma², N.G. Martin¹.* 1) Genetic Epidemiology Lab, Qld Inst Medical Res, Brisbane, Australia; 2) Psychology Dept, Free University, Amsterdam, Netherlands.

Folic acid is essential for DNA replication and cellular methylation reactions. Folate deficiency increases the risk of neural tube defects (NTD) and recurrent early pregnancy loss. Supplementation with folate around conception reduces NTD, but may increase twinning rates. A key enzyme in folate metabolism is methylenetetrahydrofolate reductase (MTHFR). Common variants at this locus result in the synthesis of an enzyme with reduced catalytic activity leading to elevated plasma concentrations of homocysteine, especially when dietary folate intake is low. Certain MTHFR haplotypes are present in fetal tissue, but not amongst neonates, suggesting that MTHFR may influence embryo survival. A recent case-control study of the C677T polymorphism found a higher frequency of the 677C allele amongst mothers of dizygotic twins (MODZT) in comparison to women who gave birth to singletons. We therefore investigated inheritance of MTHFR alleles in families of DZ and MZ twins and in families where two sisters have both given birth to spontaneous DZ twins. Allelic transmission of two common MTHFR polymorphisms (C677T and A1298C) were analyzed in 462 families of DZ and MZ twins. Allele frequencies for the 677T and 1298C alleles were 0.35 and 0.29 respectively. We also typed the variants in 454 affected sister pair families with a total of 735 MODZT. Allele frequencies for the 677T and 1298C alleles were similar in this population (0.33 and 0.31 respectively). There was strong linkage disequilibrium between the two variants and the 677T/1298C haplotype was very rare. There was statistically significant evidence of segregation distortion with reduced transmission of the 1298C allele in both groups and consequently reduced transmission of the 677C/1298C haplotype. There was no excess allele sharing among DZ twins that would be expected if MTHFR variants contributed to variation in DZ twinning. Inherited twinning was not associated with segregation of the common variants at MTHFR. The data show no association between MTHFR and DZ twinning, but some variants of MTHFR may affect embryonic survival.

Evolutionary Haplotype Linkage Disequilibrium analysis of a candidate gene for schizophrenia on 22q12-13. *F. Macciardi*^{1,2,3}, *J.R. Kim*¹, *T. Cate*¹, *A. Wong*¹, *T. Klempam*¹, *A. Macedo*⁴, *C. Pato*⁵. 1) Dept Psychiatry, CAMH, Univ Toronto, Toronto, ON, Canada; 2) Dept. of Medical Genetics, Univ of Milan, Italy; 3) GENSET S.A., Paris, France; 4) Dept. of Psychiatry, Univ of Coimbra, Coimbra, Portugal; 5) Center for Neurosciences, SUNY at Syracuse, NY, USA.

The aim of our study was to detect a possible susceptibility gene for schizophrenia on 22q12-13. Given that schizophrenia is a complex genetic trait and that consequently a susceptibility gene only conveys a small portion of the overall genetic risk, we adopted a case-control Linkage Disequilibrium strategy to detect a possible effect of such a locus, if existing. We analyzed 2 separate and independent populations, respectively from the Azores Island and Coimbra (Portugal) and Ontario (Canada): diagnosis of schizophrenia was made according to the DSM IV criteria, following a DIGS (Portugal) or a SCID-R interview. We collected 158 (P) and 189 (C) cases with matched controls. All patients and controls signed an informed consensus. We then typed 11 polymorphisms (5 microsatellites and 7 SNPs) on 22q12-13, covering a quite large area of about 5cM. As expected considering their different evolutionary history, an analysis of the marker to marker LD in both cases and controls, showed different patterns in the 2 populations, requiring an independent haplotype-disease association strategy. To detect the possible effect of an etiologic gene of small effect on schizophrenia and to reduce the high variability of the extant haplotypes, we applied a haplotype analysis by also looking at the founding haplotypes in the 2 populations separately using the program DHSMAP (Mc Peek and Strahs, 1999). Despite the haplotypes in the Portuguese and in the Canadian populations were different, reflecting their different genetic background, in both cases we obtained the same exact and significant location for a putative susceptibility gene for schizophrenia, strongly supporting the hypothesis that a true etiological genetic component for the disease exists in the area. Acknowledgment: This work has been supported by a NARSAD Independent Investigator Award to FM, 2001 Essel Investigator.

A Population Genetic Study of Autism in Andalusia, Spain. *L.A. McInnes¹, J. Hallmayer², E. Manghi³, J. Pritchard⁴, K. Wills⁵, A. Hervas⁶, S. Sanz⁷, J.L. Benitez⁷, T. Guijarro⁷, E. Gay⁷, V. Sanchez⁷, D. Llanes⁸, M. Ruiz-Rubio⁸.* 1) Dept Psychiatry/Human Gen, Mount Sinai Sch of Med, New York, NY; 2) Department of Psychiatry, Stanford University School of Medicine, Stanford CA 94304; 3) Department on Disabilities and Human Development, University of Chicago, Chicago, IL 60637; 4) 4Department of Human Genetics, The University of Chicago, Chicago, IL 60637; 5) Department of Psychology, Children's Hospitals and Clinics Minneapolis, MN 55404,; 6) Department of Child and Adolescent Psychiatry, Hospital Mutua de Terrassa, Barcelona Spain 08022; 7) Unidad de Salud Mental Infantil, Cordoba, Spain 14071; 8) Department of Genetics, University of Cordoba, Cordoba Spain 14071.

We are conducting an epidemiological and genetic study of autism in Andalusia, Spain. Andalusia harbors a population of 7 million in its 8 provinces (approximately 1.5 million are under 18) and offers several excellent advantages for such studies. Healthcare in Andalusia is coordinated by one centralized health system, the Servicio Andaluz de Salud. Medical records are centralized and virtually 100%; of cases diagnosed with autism are evaluated within this system. All children suspected of developmental delay receive Fragile X testing. We anticipate that there are up to 1500 potential cases under the age of 18 in the system. We will also collect an equal number of age and sex matched controls. Additionally, the PI of this project is organizing a genetic study of autism in the isolated population of the Central Valley of Costa Rica (CVCR). The CVCR population was largely derived from persons living in Andalusia in the 16th Century. The demographic history of Andalusia also suggests that the majority of persons now living there are descendants of the population present in the 16th Century. We will trace the genealogy of autistic probands to determine ancestry back to Andalusia at that time. We will then be able to use this subset of probands to follow up autism susceptibility loci isolated in the Costa Rican population. Our collaborators also plan to collect autistic probands from two other isolated populations founded by Andalusian immigrants.

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Novel analytical methods applied analysis Type 1 diabetes genome scan data. *F. Pociot, A.E. Karlsen, J. Nerup.*
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Background and Aims: Complex traits as T1DM are under influence of multiple genes that may interact to confer diseases susceptibility. Statistical methods in current use essentially work under single gene models, thus not allowing identification of the genetic basis of a complex trait. We therefore see an urgent need for exploring new strategies for detecting in-teracting sets of marker loci to identify interacting disease genes. **Materials and Methods:** We have chosen a combination of methods using inductive models. The methods employ spatial pattern recognition through statistical and graphical tools, in-cluding neural networks and decision tree construction, known as data-mining. These meth-ods were applied on whole-genome scan data generated on Scandinavian T1DM families (464 affected sib pairs). **Results:** In addition to the 5 regions demonstrating the highest LOD scores in classical analy-sis (HLA, IDDM15, 16p, 5p, and 2p), the present analysis identified regions on chromosomes 7, 8 and 10 to be of major importance. Interaction analysis using data mining identified the following interactions: HLA and D4S403, and HLA and D5S407 as the best predictive for T1DM, whereas the combination IDDM15 and D16S287 was the most protective, i.e. predic-tive for not having T1DM. **Conclusion:** Data-mining based methods can identify observations known from classical analysis, and may provide novel information about related interactions, which is not obtained by established methods. Finally, data mining provides also a tool for identification of protec-tive alleles.

Large variability of the FMR2 gene alleles in a normal population from Risaralda, Colombia, S.A. *L. Rengifo, J. Rodriguez, A.H. Alegria, P. Osorio, D. Gaviria, H. Serrano.* Basic Sci, Fac Med, Favi II, Univ Technologica de Pereira, Pereira, Colombia.

FRAXA and FRAXE syndromes are due to expansion of repetitive triplets. Although related by common mechanisms for expansion and abnormal methylation, they are recognized as different entities. FRAXE is considered the most prevalent cause of non specific X-linked mental retardation (FRAXE-MR) and affects at least 1/ 23.423 males. Gene FMR2 is located distally to the FRAXA region and corresponds to a folate sensitive site. FRAXE affected individuals do not present a characteristic phenotype but have slight to moderate mental retardation and other behaviour alterations. They have the FRAXA cytogenetic marker but are negative for FMR1 mutations. The FMR2 normal allele has between 3-42 CCG repeats non interrupted by AGG triplets. The limit between the normal and premutated alleles is not well defined because FRAXE full mutations are rare. Intermediate alleles have between 31-60 repeats. Affected individuals present more than 200 CCG repeats with absence or diminished synthesis of the FMR2 protein. We analyzed by PCR and Southern blot 1220 FMR2 alleles corresponding to 464 males and 378 females with ages between 18 and 81 years from a normal population in the department of Risaralda, Colombia. A large variability was found for the FMR2 allelic distribution. In the normal range (3-42 CCG repeats) we found the following groups: 2-15 repeats 137 (11.2%), 16-30 repeats 743 (60.9%) The most frequent allele had 26 repeats (203, 16.6%) followed by the one with 21 repeats (129, 15.7%). In the intermediate range (31-60 repeats) there were 340 (27.9%). The most frequent allele had 32 repeats (143, 11.7%) followed by 34 repeats (62, 5.1%). No alleles with more than 57 repeats were found. Homozygotic women were 78.5% and the most frequent genotypes were 21-21 with 55 cases and 26-26 with 46. There was no significant difference by gender.

Influence of b₂ receptor Haplotype on Obesity. *C.D. Crumel¹, J. Wang¹, H. Garg², J. Sylvester¹, J.J. Lima¹.* 1) Nemours Children's Clinic, Jacksonville, FL; 2) University of Florida, Shands Jacksonville, Jacksonville, FL.

Obesity can lead to Type II diabetes, cardiovascular disease and increased mortality. Genetic and environmental factors are known to contribute to obesity. Genes that regulate catecholamine function may be important because of the role catecholamines play in energy expenditure and lipolysis. Associations between single b₂ adrenergic receptor (b₂AR) polymorphisms and obesity have been reported in different genders of various ethnic groups. To our knowledge, there are no studies that explore associations between b₂AR haplotype and obesity. These studies are important because of the tight linkage disequilibrium between b₂AR polymorphisms. The objective of this study was to determine if the b₂AR haplotype is associated with obesity in healthy American adults. Obesity was defined as a body mass index (BMI) > 30kg/m² and a percentage of body fat (PBF) > 20% for males and > 30% for females. Final PBF was determined by averaging PBF according to skinfold thickness and PBF by bioelectrical impedance. The b₂AR polymorphisms at codons 19, Cysteine (C)/Arginine (R), 16 Glycine (G)/Arginine (R) and, 27 Glutamine (Q)/Glutamate (E) and haplotype were determined as described (Wang et al. *Am J Pharmacogenomics* 2001;1:315) in 104 obese and 89 non-obese healthy adults (average age 30 yo). The mean ± SD in BMI, PBF, fasting glucose and insulin concentrations in obese vs. non-obese were: 36±6.5 vs. 24±3.2 kg/m² (p<0.0001); 39±8.1 vs. 27±7.2 (p<0.0001); 77±26.1 vs. 75±26 mg/dl (p=0.532); 12±9.2 vs. 7.5±5.3 mIU/ml (p<0.0001), respectively. The haplotype allele frequencies were RGE 33%; CRQ, 41%; CGQ 26%. The RGE/RGE haplotype frequency was significantly higher in non-obese (20% vs. 9.6%; $\chi^2=4.24$, p=0.037) compared to obese individuals. No other significant association between the frequencies of haplotype pairs and obesity was observed. Stratification by gender and race resulted in insufficient numbers for statistical analysis. Larger numbers are required to determine if the association between RGE haplotype and obesity is dependent on gender and race. Conclusion: The b₂AR RGE haplotype may have a modest protective effect against obesity in healthy American adults.

Heritabilities of Biochemical Urine Components in Families with Nephrolithiasis. *I.P. Kovac¹, A.F. Wilson¹, J. Kinder², B.J. Coe², F.L. Coe³, J.R. Asplin².* 1) Genometrics, NHGRI, Baltimore, MD; 2) Litholink Corporation, Chicago, IL; 3) University of Chicago, Chicago, IL.

Nephrolithiasis is a common disorder with a lifetime prevalence in the United States of 10% in men and 4% in women. Stone formation is familial but also affected by diet and environment. Our objective was to estimate the heritabilities of quantitative measurements associated with kidney stone formation to determine their suitability for further genetic studies. Seventeen biochemical traits were measured in 877 individuals from 242 families. Data were analyzed as averages from two 24h urine collections, except for 68 individuals for which only one urine collection was obtained and included as such. Biochemical measurements included: urine volume, citrate, calcium, oxalate, uric acid, sodium, potassium, phosphorus, magnesium, creatinine, ammonium, sulfate, pH, and urea nitrogen. Estimates of heritabilities and 95% confidence intervals were obtained using age-adjusted regression of offspring on mid-parental biochemical trait values, computed on 181 observations. Excretion rates for all chemistries were adjusted for creatinine excretion to compensate for variability in completeness of collection in duplicate urine collections. Highest heritabilities were for creatinine (0.54, 95% CI = 0.17 - 0.90), uric acid supersaturation (0.45, 95% CI = 0.26 - 0.64), calcium/creatinine ratio (0.43, 95% CI = 0.22 - 0.64), calcium oxalate supersaturation (0.40, 95% CI = 0.15 - 0.65), and urine volume (0.39, 95% CI = 0.18 - 0.60). Moderate heritabilities were also observed for oxalate/creatinine ratio (0.38, 95% CI = 0.12 - 0.64), uric acid/creatinine ratio (0.34, 95% CI = 0.14 - 0.54), phosphorus/creatinine ratio (0.33, 95% CI = 0.07 - 0.59), urine pH (0.29, 95% CI = 0.13 - 0.46), and potassium/creatinine ratio (0.24, 95% CI = 0.03 - 0.44). We conclude that several phenotypes in our data set, particularly those with higher estimated heritabilities, may represent quantitative phenotypes suitable for further studies focusing on the detection and localization of genes influencing the pathogenesis of kidney stones.

Mitochondrial DNA sequence variations of five ethnic populations in northern China. *Q.-P. Kong¹, Y.-G. Yao¹, M. Liu², S.-P. Shen², C. Chen², C.-L. Zhu¹, M.G. Palanichamy¹, Y.-P. Zhang¹.* 1) Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan, 650223 China; 2) Department of Pathology, Baotou Medical College, Baotou, Inner Mongolia, 014010 China.

To study the mitochondrial DNA sequence variations in a total of 232 individuals from five ethnic populations (Ewenki, N=47; Korean, N=48; Mongolian, N=48; Oroqen, N=44; Daur, N=45) in northern China, we analyzed the control region and typed for a number of characteristic mutations in coding regions by direct sequencing or restriction-fragment-length-polymorphism tests. With the exception of several individuals belonged to the European specific haplogroup J, which might reflect a recent gene flow from Europe, most of the individuals considered can be assigned into the east Asia specific haplogroups as reported in the recent studies. The distribution frequencies of haplogroups varied in the five ethnic populations. Haplogroup B had the highest frequency in Daur (17.8%), followed by Korean (16.7%). However, in Oroqen, only one individual belonged to haplogroup B. The frequency of haplogroup D was more than 31% in the four populations except for that of Daur (24.5%). The Daur sample also contained a high amount of M7 types (20.0%), which were either absent or with low frequency in other populations. Haplogroup A had the highest frequency in Korean (14.6%), while in the other populations, the frequency was lower than 8.5%. Haplogroup C constituted a main genetic component of Oroqen (29.5%). Generally, Ewenki and Oroqen, Mongolian and Korean showed closer genetic relationship in the principle component map based on the haplogroup frequency matrix, whereas Daur occupied a relative remote location in the map. The lower genetic diversities observed in Ewenki and Oroqen were also consistent with their small population census sizes.

Specific mitochondrial haplogroups affect risk for late-onset Parkinson disease. *J.M. Van Der Walt^{1,2}, K.K. Nicodemus^{1,2}, E.R. Martin^{1,2}, W.K. Scott^{1,2}, M.A. Nance², J.P. Hubble², J.L. Haines², W.C. Koller², R. Pahwa², M. Stern², B.C. Hiner², J. Jankovic², C.G. Goetz², G.W. Small², F. Mastaglia², R.A. Gibson², L.T. Middleton², A.C. McLaurin^{1,2}, M.A. Pericak-Vance^{1,2}, J.M. Vance^{1,2}.* 1) Ctr Human Genetics, Duke Univ, Durham, NC; 2) Duke CHG/GlaxoSmithKline PD Genetics Collaboration.

Evidence suggests that mitochondrial (mt) impairment contributes to the pathogenesis of Parkinson disease (PD). We tested whether single nucleotide polymorphisms (SNPs) and mt haplogroups confer risk to late-onset PD in a Caucasian sample consisting of 627 cases and 353 controls. Ten stable, ancestral SNPs were genotyped by the allelic discrimination method (Taqman). Combinations of SNPs were used to classify each individual into a European haplogroup (H, I, J, K, T, U, V, W and X). Overall, individuals classified as haplogroup J (OR=0.56; 95% CI, 0.34 to 0.91; p=0.02) or haplogroup K (OR=0.52; 95% CI, 0.31 to 0.86; p=0.01) demonstrated a significant decrease in risk of PD versus individuals carrying the common haplogroup H. Furthermore, a specific SNP that defines these haplogroups, 10389G, strongly contributes to this overall protective effect (OR=0.55; 95% CI, 0.41 to 0.75; p=0.0001). The protective effect of the J haplogroup is interesting given the recently published association between J and longevity. Stratification by gender revealed that males classified as haplogroup U showed an increase in risk of developing PD (OR=2.2; 95% CI, 1.09 to 4.43; p=0.03) when compared to all other males classified as haplogroup H. This result is compelling since haplogroup U is also a risk factor for Alzheimer disease in males (Pericak-Vance et al., ASHG 2002). These findings demonstrate that the closely related K and U haplogroups have markedly different effects on risk of developing late-onset neurological diseases. The difference in risk between these haplogroups can be explained by the presence of the SNP 10398G in K and J and its absence in haplogroup U. We have revealed that mt variability affects risk for late-onset PD and suggest that males of a certain mitochondrial lineage may be at greater risk.

Frequency of deletions in dystrophin gene in Mexican patients with muscular dystrophy Duchenne/Becker and carrier status detection. *G. Garcia-Escalante¹, L. Gonzalez-Herrera¹, I. Castillo-Zapata¹, D. Pinto-Escalante¹, A. Gonzalez-Del Angel².* 1) Lab de Genetica, Univ Autonoma Yucatan, Merida Yucatan, Mexico; 2) Departamento de Investigacion en Genetica Humana INP Mexico, DF:.

Muscular dystrophy Duchenne and Becker are allelic disorders with an X linked recessive pattern of inheritance, caused by mutations on the dystrophin gene (Xp21.2). Most frequent mutations are intragenic deletions of one or more exons 50-70%, intragenic duplications in 6-8% while the remaining are point mutations and others. One third of patients have new mutations. Detection of carrier status is very important for diagnosis and genetic counselling in DMD/DMB to prevent the disorder. We determined the frequency of deletions in dystrophin gene in Mexican DMD/DMB patients and identified the carrier status in familial cases. Detection of deletions was carried out in 11 subjects with a clinical diagnosis of DMD and their families, using Multiplex-PCR of 22 segments included in 4 series. Analysis of intragenic RFLPs markers pERT 87.8/TaqI, intron 13, pERT87.15 /Bam/Hi intron 15, y the VNTR MP1P UTR 3' were used for the determination of carrier status on the basis of segregation analysis. The 81% (9/11) were sporadic familial cases. The 64% of the mutations detected in patients were deletions localized in major hotspot region (44-52 exons). 86% of the mothers were informative for at least one polymorphic marker. Carrier status was identified in 84% (11/13) of the sisters and it was discarded in two of them. The allele frequency of polymorphic markers and heterozygosity index found in the studied population were: 0.61 allele A2 (74+71 pb) 87.8/Taq I and 0.91 allele F2 (166+50 pb) pERT 87.15/BamHI and 0.42 and 0.17 of heterozygosity index for each one. The frequency of deletions in the dystrophin gene obtained in these Mexican patients (64%) correlates with the frequency reported for populations worldwide. Detection of mutation and identification of carrier status will provide DMD families accurate genetic counselling.

Molecular evidence for the paternal age effect in sperm. *R.L. Glaser, R.L. Schulman, E.W. Jabs.* Inst. Genet. Med, Johns Hopkins Univ., Balto., MD.

More than 10 genetic disorders are associated with advanced paternal age; half result from mutations that are exclusively paternal in origin. The paternal age effect could be explained by the copy-error hypothesis. In males, 36 divisions give rise to the spermatogonia stem cells that are present before puberty. After puberty, these cells divide every 16 days or 23 times per year. Because replication of spermatogonia is constant during a man's lifetime, the number of mutations could increase or accumulate with age. To test this hypothesis we collected sperm samples from men who were recruited using a questionnaire designed to exclude unusual exposures. Samples were screened for the two FGFR2 mutations accounting for 99% of all cases of Apert syndrome because they are associated with advanced paternal age, exclusively paternal in origin, recurrent and relatively frequent. The more prevalent mutation, 934C→G, occurs within a CpG dinucleotide while the 937C→G mutation does not. We developed allele-specific peptide nucleic acid mediated PCR-clamp methods with the Roche LightCycler that were robust, specific and sensitive enough to detect one mutant sperm per 20,000. Samples from 59 men ages 20-79 were analyzed (13 had fathered a child with sporadic Apert syndrome). Mosaicism was also assessed by analyzing a second sperm sample and a blood sample from a subset of positive individuals. In 46% (27/59) of the men either one or both mutations were detected in their sperm but were not present in their blood. Among the men who had not fathered a child with Apert syndrome, the percentage with the 937C→G mutation increased with age (17%, ages 20-29 vs 60%, ages 70-79). The frequency of this mutation in sperm also increased with age. The average number of mutant sperm per million in men ages 20-29 was 0.044 ± 2.08 as compared to 10.73 ± 3.83 in men ages 70-79 ($p < 0.01$). The frequency of the 934C→G mutation did not increase with age. However, the fathers of Apert children had 2.09 ± 0.87 more mutant sperm per million with the 934C→G mutation as compared to their age matched cohort ($p < 0.01$). Our data provides molecular evidence in support of the paternal age effect and suggests that different types of mutations may have different frequencies in sperm.

Identification of new mutations within ARS gene in MDM Tunisian patients. *C. Charfeddine¹, S. Abdelhak¹, M. Mokni², S. Boubaker³, S. Ghedamsi², R. Elkares¹, A. Ben Osman², K. Dellagi¹.* 1) Immunology, Institut Pasteur, Tunis, Tunisia; 2) Service de Dermatologie, Hôpital de La Rabta, Tunis, Tunisia; 3) Service Anatomopathologie, Institut Pasteur, Tunis, Tunisia.

Mal de Meleda (MDM) is a rare autosomal recessive genodermatosis with a prevalence in the general population of 1 in 100 000. This skin disorder is characterized by symmetric, transgressive palmoplantar keratoderma (PPK), keratotic skin lesion and hyperhidrosis. It can be associated with brachydactyly and nail abnormalities. The gene responsible for MDM has been mapped on chromosome 8qter by homozygosity mapping in large consanguineous Algerian families and coding a protein named SLURP-1, for secreted Ly-6/uPAR related protein-1. Mutations have been shown to be involved in MDM. The ARS gene extends over 2 Kb and comprises 3 exons. Three different homozygous mutations 82delT, 178G+1A and C286T were detected in families of Algerian and Croatian origin. Genetic analysis revealed three different haplotypes cosegregate with these mutations. Our aim study was the investigation of the molecular basis of MDM in Tunisian patients. Mutation screening of ARS gene within 8 consanguineous Tunisian families from different geographical origins, including 12 affected individuals, lead to the identification of three different ARS alteration. The 82delT mutation, identified within Algerian and Croatian patients was found in 8 patients belonging to 4 families. Two new mutations specific to Tunisian patients were identified, these mutations are missense C99Y and C77A within exon 3.

Mutation in the *PAX9* gene and absence of mutations in *MSX1* gene in Portuguese families with hypodontia.

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Hypodontia, the congenital absence of one or several permanent teeth, represents one of the most common forms of dental anomalies. Although the molecular mechanisms involved in tooth development are largely unknown, homeobox genes are known to be related to tooth genesis and orofacial clefting. Two genes, *PAX9* and *MSX1*, have been implicated in the agenesis of the second premolars and third molars and in the lack of most permanent molars, respectively. *MSX1*, however, has been excluded in families with other forms of specific hypodontia, such as the lack of incisors and premolars. We have studied the occurrence of congenitally missing permanent lateral incisors in a sample of 16,771 patients, registered at a university dental clinic, for whom ortopantomographies were available. In 219 patients, congenital absence of maxillary incisor was observed and confirmed by questionnaire/observation, corresponding to a prevalence of 1.3%. Thirty-four of these probands were observed clinically, as well as their direct relatives and from these, 17 (50%) reported a familial history of tooth absence and/or anomalous tooth structure. We performed mutational analysis of the coding region (exons 2 to 4) of *PAX9* and of the coding region of *MSX1* (first and second exons), in probands from twelve Portuguese families, comprising 23 cases of hypodontia and 24 healthy individuals. The direct sequencing of PCR products containing the exons and intron/exon borders of these genes showed two nucleotide changes in exon 3 of *PAX9* gene: a silent change (C717T) in His239 codon in the homozygous state was present in one family, while a missense mutation (G718C), causing an Ala240Pro substitution, was observed in 5 families, both in the homozygous and heterozygous state. This study will proceed with analysis of other members of these families, to determine the correlation between the referred mutation and the observed phenotypes. No nucleotide changes were observed in the *MSX1* gene.

Double Mutations Can Cause False Exclusions in STR Paternity Testing. *G. Sun¹, S. Raskin², J. Sun¹, H. Cheng¹, A. Deka¹, R. Chakraborty¹, R. Deka¹.* 1) Dept Environmental Health, Univ Cincinnati, Cincinnati, OH; 2) Laboratório Genética, Curitiba, Brazil.

PCR-based typing of STR loci is currently the most widely used genetic tool for paternity testing. However, as yet the analytical treatment of mutations at such analyses is not a fully resolved issue. Mutation rate at most tetra-nucleotide loci is low, and consequently, the chance of two paternal mutations is low. Therefore, it is accepted that nonpaternity can be asserted when at least two exclusions are found. In the analysis of 13 CODIS STR loci involving the mother, child, and alleged father, one mismatch between the alleged father and child is a single exclusion. Non-paternity cannot be determined without typing additional loci. Such tests will reveal either: more loci excluding the alleged father; or no further exclusion, indicating that the original single exclusion is a mutational event. In a dataset of 3322 trios with disputed paternity, 77 single exclusionary cases were found to be mutational events. In addition, for the 6 double exclusionary cases, we analyzed nine supplementary STR loci. Only one case was found to be real exclusion, confirmed by six exclusions; the other five double exclusionary cases could not be excluded, establishing these to be double mutational events. The observed probability is 0.15%, indicating that double mutations may be observed three times in two thousand trios. These observations imply that all two-locus exclusionary events are not necessarily due to non-paternity. In our data, the observed average mutation rate is 1.6×10^{-3} , with a 10-fold bias of paternal versus maternal mutations. So, when 13 STR loci are tested, the expected frequencies of one-locus and two-locus paternal mutations would be 3.15%, and 0.099%, respectively, translating in an occurrence of one double mutation event in every one thousand tests. We observed 94% of the mutations are loss or gain one repeat, and only 6% involving two or more repeats. Thus, repeat number differences of discordant alleles at the exclusionary loci should also be considered along with typing a supplementary set of loci to distinguish double mutation events from actual nonpaternity.

An analysis of OPTN gene mutations among Japanese patients with normal tension glaucoma and primary open-angle glaucoma. *S. Tang¹, Y. Toda¹, D.R. Smith¹, F. Mabuchi², Z. Yamagata¹*. 1) Health Sciences, Yamanashi Medical University, Yamanashi, Japan; 2) UCSD Shiley Eye Center, La Jolla, California.

Glaucoma represents one of the most common eye diseases and is characterized by progressive loss of visual acuity. In the more advanced stages, bilateral blindness may result due to optic nerve atrophy and an excavated optic nerve head. Open-angle glaucoma represents one of the main disease subsets, which may be further divided into primary open-angle glaucoma (POAG) and normal tension glaucoma (NTG). The optic neuropathy inducing gene (OPTN) has been previously identified as a causative factor for POAG. To investigate possible racial differences, single-strand conformation polymorphism analysis and subsequent sequence analysis was performed for genotyping the OPTN gene in 148 unrelated Japanese patients with NTG, 165 patients with POAG and 202 controls who were not suffering glaucoma. Although c.412G>A was found in all NTG, POAG and controls, we were unable to detect any c.458G>A mutation among the Japanese patients. Furthermore, there were no significant differences between NTG / POAG and control groups. Interestingly, the novel c.457C>T (T49T) genotype was found in one POAG patient. Our study suggests that there may be certain racial differences between Japanese and Caucasians with respect to OPTN genotypes.

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Genetic Polymorphism of N-acetyltransferases 1 in a Korean Population. *S.Y. Kong, J.W. Kim.* Clinical Pathology, Samsung Medical Center, Seoul, Korea.

N-acetyltransferase isozymes, NAT1 is polymorphic and capable of N-acetylation of aromatic and heterocyclic amine carcinogens. Ethnic differences exist in NAT1 genotype frequencies that may be a factor in cancer incidence. But, less work has been done to characterize NAT1* allelic variants in Korean population. A group of 100 unrelated Korean subjects was genotyped using a polymerase chain reaction and direct sequencing. A entire 872 base pair coding region of NAT1 gene was fully sequenced. Of 200 NAT1*alleles assayed, 57% were NAT1*4 wild type. Alleles NAT1*10 and *3 were found at 39.5% and 3.5%, respectively. Major genotypes were NAT1 *4/*10(45%), *4/*4 (31%) and *10/*10(17%). The unusually high frequency of NAT1*10 alleles, which contains a variant polyadenylation signal may be significant for epidemiological studies of the effects of the NAT1 polymorphism in Korean population.

Haplotype analysis of non-response to antihypertensive medication and predicting progression to renal failure in the AASK trial. *M.O. Skidmore¹, M.S. Lipkowitz⁴, B.K. Rana¹, M.G. Robinson¹, P. Mahbouti², G. Zhang¹, D. Smith³, D.T. OConnor², N.J. Schork¹ and The UCSD/Sequenom Autonomic Pharmacodynamic Pharmacogenomics Program Project.* 1) Dept. of Psychiatry, UC San Diego, School of Medicine, La Jolla, CA; 2) Dept. of Medicine, UC San Diego, School of Medicine, La Jolla, CA; 3) Dept. of Biology, UC San Diego, La Jolla, CA; 4) Dept of Medicine, Mount Sinai School of Medicine, New York, NY.

The African American Study of Hypertension and Kidney Disease (AASK) trial¹ conducted from 1995 to 2000 examined 1094 African American patients with hypertensive renal disease and compared metoprolol, ramipril, and amlodipine for efficacy with respect to blood pressure, end stage renal failure (ESRF), and mortality. Patients were followed for three to four years and changes in their blood pressure, urinary protein to creatinine ratio, and glomerular filtration rate were assessed. We have genotyped over 300 trial patients using SNPs in genes in the renin-angiotensin-aldosterone (RAS) system, beta adrenergic receptors and ion channels. SNPs were used to infer phased haplotypes with the program SNP-EM. These SNPs were also used to assess population specific substructure² using the program STRUCTURE. Our results suggest that haplotypes analysis in a heterogeneous cohort can be used to evaluate efficacy in a clinical trial. These results will further refine the development of molecular diagnostic assays for internists and nephrologists managing hypertensive patients with renal disease.

¹ Agodoa LY, Appel L, Bakris GL, *et al.* Effect of ramipril vs amlodipine on renal outcomes in hypertensive nephrosclerosis: a randomized controlled trial. *JAMA.* 2001 Jun 6;285(21):2719-28.

²Wilson JF, Weale ME, Smith AC, Gratrix F, Fletcher B, Thomas MG, Bradman N, Goldstein DB. Population genetic structure of variable drug response. *Nat Genet.* 2001 Nov;29(3):265-9.

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Latent class analysis does not support migraine with aura and migraine without aura as separate entities. *D.R. Nyholt¹, N.G. Gillespie¹, A.C. Heath², K.R. Merikangas³, D.L. Duffy¹, N.G. Martin¹.* 1) Genetic Epidemiology Lab, QIMR, Herston, QLD, Australia; 2) Department of Psychiatry, Washington University School of Medicine, St Louis, Missouri; 3) Yale University School of Medicine, Genetic Epidemiology Research Unit, New Haven, Connecticut.

To examine whether or not migraine without aura (MO) and migraine with aura (MA) are two separate entities, latent class analysis (LCA) was used to investigate the presence and composition of migraine symptom subgroups in a community sample of 6265 Australian twins (55% female) aged 25-36 who had completed an interview based on International Headache Society's (IHS) criteria. Consistent with prevalences in similar populations, 605 (17.5%) female and 212 (7.6%) male twins satisfied the IHS criteria for migraine without aura.

Latent class analysis of IHS symptoms identified three major symptom classes, representing i) a mild form of recurrent, typically pulsating, non-migrainous headache, ii) a moderately severe form of migraine typically, but not always, *without* visual aura (i.e., 41.9% individuals positive for aura), loading on all IHS MO symptoms except "unilateral location" and "nausea, vomiting or diarrhoea", and iii) a severe form of migraine typically *with* visual aura (i.e., 76.3% individuals positive for aura), loading on all IHS symptoms for migraine.

Genetic model fitting indicated a significantly greater genetic contribution to migraine using the LCA classification (heritability, $h^2=0.44$, 95% CI: 0.38-0.51) compared to the IHS classification ($h^2=0.33$, 95% CI: 0.22-0.43). Importantly, factor analysis indicated a single factor best fit the data and therefore does not support the hypothesis of MO and MA as separate subtypes. Rather, our data indicate the existence of a continuum of severity.

In searching for predisposing genes, we should therefore expect to find some trait loci in common to all major classes of migraine, with perhaps additional loci, or more severe alleles associated with more severe types, including MA.

Folic acid fortification decreases the rate of Spina Bifida (SB) in an inner city population by 65%. *R.W. Marion^{1,2}, L. Schendel², E. Hantman^{1,2}*. 1) Children's Hospital at Montefiore, Bronx, NY; 2) Blythedale Children's Hospital, Valhalla, NY.

In 1992, the U.S. Public Health Service recommended that to reduce the risk of neural tube defects, women should take 0.4 mg of folic acid prior to conception. In 1998, by order of the FDA, fortification of staple foods with 140 ug/100 g was begun. In order to assess the effect of these recommendations on the incidence of SB, we analyzed the year of birth of all 204 patients registered for care at the SB Clinic (SBC) at Blythedale Children's Hospital since the clinic's inception in February 1987. The clinic serves an inner city population. Since 1987, no changes have occurred in prenatal diagnostic availability, incidence of elective TOP or referral patterns. All babies with SB discharged from our affiliated hospitals are referred to our clinic. Our data show that from 1987 through 1992, an average of 9.3 new infants per year were enrolled in the SBC. From 1993-1998 (the years following the Public Health Service's recommendation), the average dropped slightly to 8 infants/yr. In 1999-2001, following mandated fortification of staple foods, only 10 new pts were born, an average of 3.3 pts/year. Our data indicate that, although passive recommendations about folic acid use had little effect on the incidence of SB in an inner city population, active fortification of the food supply decreased the number of children entering our SBC by nearly 2/3. This figure correlates well with observations from other populations.

A TNF- α inducible promoter variant of interferon-gamma accelerates AIDS progression. *P. An*¹, *D. Vlahov*², *J.B. Maroglick*², *J. Phair*³, *T.R. OBrien*⁴, *J. Lautenberger*⁵, *S.J. OBrien*⁵, *C.A. Winkler*¹. 1) IRSP, SAIC-Frederick, Inc., NCI Frederick, Frederick, MD; 2) Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 3) Northwestern University Medical School, Comprehensive AIDS Center, Chicago, IL; 4) Viral Epidemiology Branch, DCEG, National Cancer Institute, Bethesda, MD; 5) Laboratory of Genomic Diversity, National Cancer Institute, Frederick, MD.

Polymorphisms in the genes for HIV-1 coreceptors and their natural ligands have been shown to modify HIV-1 transmission and disease progression and have led to important insights into the pathogenesis of HIV-1 infection. Cytokine genes, having a broad role in immune regulation, are candidate genetic factors that may modify HIV-1 pathogenesis. Interferon-gamma (IFN-gamma), is a crucial regulator of cellular immune responses to intracellular pathogens. We recently reported a single nucleotide polymorphism, IFNG -179G/T, in the promoter of IFNG that the variant allele is present in 4% of African Americans and less than 0.2% of European Americans. The IFNG promoter carrying the variant allele -179T was shown to be inducible by TNF- α and to constitutively bind nuclear extracts obtained from T cells, while the promoter with the more frequent -179G allele is non-responsive to TNF- α . We thus investigate the effects of IFNG -179G/T on AIDS progression in HIV-1 infected individuals. In African American HIV-1 seroconverters, the IFNG -179G/T heterozygous genotype was found to be associated with accelerated rate of progression to CD4⁺ T cell < 200 cells/mm³ and AIDS (1993 CDC definition). Our results show that IFNG -179T associated with altered regulation of gene transcription is a risk factor for progression of HIV-1 disease. It is possible that TNF- α induces increased IFN-gamma production when the -179T allele is present, and together these two cytokines act synergistically to induce CD4⁺ T cell depletion by apoptosis. It remains to be determined if the -179T allele is associated with susceptibility to other infectious diseases or immune disorders, particularly those disproportionately affecting people of African origin or descent. (Funded in part by DHHS #NO1-CO-12400.).

Association between the ABCA1/G1051A polymorphism and Alzheimers disease. *P.P. Desai*¹, *S.T. DeKosky*^{1,2,3}, *M.I. Kamboh*^{1,2}. 1) Human Genetics, Univ Pittsburgh, Pittsburgh, PA; 2) Psychiatry, Univ Pittsburgh, Pittsburgh, PA; 3) Neurology, Univ Pittsburgh, Pittsburgh, PA.

Alzheimers disease (AD) is a genetically complex neurodegenerative disorder that results in the impairment of multiple cognitive functions. Increasing evidence suggests a potential role of cholesterol in the pathophysiology of AD, and provides a rationale for examining the role of genes involved in cholesterol metabolism in AD pathogenesis. The ATP-binding cassette transporter 1 (ABCA1) regulates cholesterol efflux from tissues into the reverse cholesterol transport pathway. Thus, ABCA1 activity is crucial for the excretion of cellular cholesterol and for the synthesis of high-density lipoproteins. ABCA1 is also a positional candidate gene as it is located on human chromosome 9q22-q31, which is in close vicinity to the 9q34 region linked to AD in previous studies. Several polymorphisms have been identified in the ABCA1 gene, which have been associated with coronary artery disease or Tangier disease. No genetic association studies have yet been carried out to investigate the role of ABCA1 in AD. In the present study, we examined the role of the ABCA1/G1051A polymorphism in modifying the risk of AD in 693 sporadic, late-onset AD cases (mean age $72.94 \pm \text{SD } 6.82$) and 645 non-demented controls (mean age $75.89 \pm \text{SD } 5.08$). The G1051A variant showed a modest association with AD, with an age, gender and APOE-adjusted OR of 1.30 (95%CI: 1.02-1.66; $p=0.036$). When the data were stratified by gender, significantly higher frequencies of 1051*A allele (0.308 vs. 0.255; $p=0.014$) and 1051*A allele carriers (54.8% vs. 43.7%; $p=0.001$) were observed among female AD cases ($n=469$) compared to female controls ($n=396$), with an adjusted OR of 1.68 (95%CI: 1.24-2.28; $p=0.0009$). When the female data were further stratified by APOE*4 carrier status, the G1051A polymorphism showed a significant association with AD among non-APOE*4 carriers (OR 1.74; 95%CI: 1.20-2.52; $p=0.0036$). Our data confirm the existence of a putative AD risk gene in the chromosome 9q region, and suggest gender differences in that risk.

alpha 2b adrenoceptor polymorphism and Cardiovascular disease in Koreans. *J.R Choi^{1,2}, E.Y Cho^{1,2}, S.J. Bae^{1,3}, H.Y. Park^{1,2}, Y. Ko^{1,2}, Y. Jang^{1,2}.* 1) Cardiovascular Genome Center, Yonsei University, Seoul, Korea; 2) Yonsei Cardiovascular Research Institute, Division of Cardiology, Department of Internal Medicine, Yonsei University, College of Medicine, Seoul, Korea; 3) Department of Food and Nutrition, College of Human Ecology, Yonsei University, Seoul, Korea.

Cardiovascular disease is a complex multigenic disorder, which the significant inheritable element have important roles with environmental factors. Recently significant evidence has been provided for the pathophysiological involvement of the alpha 2 adrenoceptor gene in coronary artery disease. alpha 2-adrenoceptor subtype, in particular the 2b subtype, may also play an important role in the vasoconstrictor responses to the alpha 2-adrenoceptor agonists. The aim of this study was to investigate the relationships between an insertion/deletion(I/D), 9 base size difference polymorphism in the 2b-adrenoceptor gene and cardiovascular diseases and blood pressure in Korean male subjects. We examined alpha-2b adrenoceptor polymorphism which was located in the third intracellular loop of the receptor polypeptide, in 134 patients with myocardial infarction and 94 healthy males who visited the cardiovascular genome center in Yonsei Medical Center. In patient group, 53 men (0.40) had the I/I genotype; 54 men (0.40) had a heterozygous genotype, and 27 (0.20) had the D/D genotype. In control, 30 of 94 men(0.32) had the I/I genotype; 52(0.55) had the I/D genotype and D/D genotype frequency was 0.13. D allele frequency in MI patients was slightly higher than its in controls, but was not significantly different from two groups. alpha-2b adrenoceptor D polymorphism was not affected to systolic and diastolic blood pressure in healthy group. According to the logistic multiple regression test, the alpha 2-adrenoceptor genotype was not significantly associated with coronary artery disease in this study groups(OR=1.724 CI:0.824-3.608). The alpha 2-adrenoceptor gene is not a novel risk factor for coronary artery disease in Koreans and it was a different results in Finnish men.

Guanine nucleotide binding 3 (GNB3) haplotype analysis in the Venecia-Colombia Latin-American blood pressure study. *E.A. Garcia*^{1,2}, *D. Aristizabal*³, *J. McEwen*², *J. Mendez*², *P. Munroe*¹, *M. Caulfield*¹. 1) Clinical Pharmacology, St Bartholomews, London, Central london, England; 2) Corporacion para Investigaciones Biologicas CIB, Seccion de Biologia Molecular. Medellin-Colombia; 3) Department of Preventive Medicine. Clinica Medellin. Medellin-Colombia.

Blood pressure is a continuous variable and the upper extreme of the distribution is an independent risk factor for cardiovascular disease. Polymorphisms in GNB3 have been associated with essential hypertension in some genetic studies. Our aim was to test if GNB3 was associated with hypertension in individuals of Latin-American descent. One thousand and two healthy individuals from Venecia-Colombia were recruited in 1997 to study the genetic component of blood pressure. A diastolic population blood pressure distribution was constructed. Individuals in the upper and lowest quintile were studied: hypertensive (HT) and normotensive (NT) groups. Five SNPs: A-350G in the promoter and 4 in the gene T657A, G814A, C825T, and C1429T were genotyped for both groups using the SNP scoring platform of PyrosequencingTM. One hundred and thirty-two hypertensives and 160 NTs were genotyped for all SNPs. Hardy Weinberg Equilibrium was found for all the SNPs typed. The allele frequencies were not found to differ between HT and NT groups. Twelve haplotypes were found in 584 chromosomes, 4 haplotypes represented 68% of the population. The 4 common haplotypes were (HT frequencies-NT frequencies): hap4 GTGTT 0.28-0.31; hap2 GTGTC 0.16-0.12; hap5 GTGCC 0.30-0.33 and hap1 ATGCC 0.12-0.14, none were associated with hypertension ($P > 0.05$). In summary, no association was found between the variants analyzed in GNB3 and blood pressure. Power calculations with the observed allele frequencies suggest that more individuals are required before GNB3 can be excluded. At present we are genotyping the whole Venecia population to establish if this gene is associated with normal variation in blood pressure levels.

LACK OF ASSOCIATION BETWEEN ANGIOTENSIN I CONVERTING ENZYME GENE POLYMORPHISM AND DIABETIC NEPHROPATHY IN TUNISIAN POPULATION. *I. Arfa¹, C. Amrouch-Rached², A. Abid², S. Blousa-Chabchoub², S. Chakroun¹, K. Dellagi¹, S. Abdelhak¹.* 1) Immunology, Institut Pasteur, Tunis, Tunisia; 2) Institut national de nutrition ,Tunis ,Tunisia.

Diabetic nephropathy is a micro vascular complication of type 1 and type 2 diabetes due to uncontrolled diabetes and genetic susceptibility factors. It can be caused by an increase of glomerular pressure due to diabetes. The glomerular pressure is controlled by the renin-angiotensin system namely angiotensin II and angiotensin I converting enzyme (ACE). The gene encoding ACE has an insertion/deletion(I/D) polymorphism that appears to be associated with diabetic nephropathy. The aim of our study was to investigate the relationship of this polymorphism and diabetic nephropathy in the Tunisian population. A case-control study was conducted among 55 diabetic patients with nephropathy complication ,53 diabetic patients without diabetic nephropathy and 71 healthy controls. Genotyping was performed using a nested PCR amplification in order to identify correctly heterozygotes individuals. Our results indicate that the distribution of DD, ID and II genotypes of the ACE gene did not differ among the three groups. The present study shows that there is no association between ACE I/D polymorphism and diabetic nephropathy in the Tunisian population.

Interaction of C677T and A1298C polymorphisms in the MTHFR gene in association with neural tube defects in the State of Yucatan, Mexico. *L.J. Gonzalez-Herrera¹, M.P. Flores-Machado¹, I.C. Castillo-Zapata¹, M.G. Garcia-Escalante¹, D. Pinto-Escalante¹, A. Gonzalez-Del Angel².* 1) Genetics Lab, CIR, Univ Autonoma de Yucatan, Merida, Yucatan, Mexico; 2) Departamento de Investigacion en Genetica Humana. INP.

Neural tube defects (NTD) are highly prevalent (6.6 in 1 000 births) in the State of Yucatan, Mexico. Several studies have demonstrated that the two common polymorphisms, C677T and A1298C in the MTHFR gene might be associated with NTD in some populations. Compound heterozygosity CT/AC accounts for a proportion of folate related NTDs and might be associated with an increased risk for spina bifida in comparison with the presence of two wild-type alleles. In order to determine the role of these two polymorphisms and their interaction in the aetiology of NTD in the State of Yucatan, we genotyped 74 subjects with NTD and 102 NTD parents to compare with 110 control subjects. Both polymorphisms were determined by PCR-RFLP's. Allelic, genotypic and haplotypic frequencies for both polymorphisms were obtained in each group. Genotypic frequencies in control group for both polymorphisms were according to Hardy-Weinberg expectations ($p > 0.33$). We did not find any statistically significant difference in frequencies for the two polymorphisms between cases and controls ($p > 0.05$). Additionally, we made a comparison on the basis of the gender to distinguish the contribution of mothers and fathers. NTD mothers were compared with control mothers and NTD fathers with control fathers. Interestingly, we found a statistically significant differences in the mothers for allele 1298C ($p = 0.025$; OR 2.91 IC 1.02-8.68), for the heterozygous AC genotype ($p = 0.046$; OR 2.78 IC 0.90-8.88) and for the double heterozygous haplotype CT/AC ($p = 0.054$; OR 4.0 IC 0.68-26.19). Results suggest that the C677T and A1298C variants in MTHFR gene are not associated with NTD neither in the affected subjects nor in the fathers with NTD, whereas for mothers, the A1298C allele, AC genotype and AC/CT are associated with NTD. So, these genetic features are maternal risk factors for having NTD offspring in the studied population of the State of Yucatan, Mexico.

Effects of additive selection and recombination on Homozygosity Test at microsatellite loci under generalized stepwise mutation model. *Y.-X. Fu¹, H. Xu¹, M. Kimmel², A. Renwick², R. Chakraborty³*. 1) Human Genetics Center, Univ. Texas School of Public Health, Houston, TX; 2) Statistics Department, Rice University, Houston, TX; 3) Center for Genome Information, Department of Environmental Health, Univ. Cincinnati, Cincinnati, OH.

The conditional distribution of expected homozygosity in a sample given the number of segregating alleles has been widely used as a test of selective neutrality for protein and immunological markers. The underlying theory is that the allele frequency spectrum observed in a sample for a fixed number of observed alleles is independent of the parameters of the allele frequency distribution. Thus, the critical region of the test procedure can be evaluated from the knowledge of sample size (n) and number of alleles (k). Analytical proof of this theory holds for a population of constant size under mutation-drift equilibrium with the Infinite Allele Model (IAM) of mutations. However, theoretical as well as empirical data demonstrate that microsatellite loci evolve with stepwise mutations (SMM) where the repeat size changes induced by single mutations are restricted (most often increase/decrease of one repeat unit). We have shown that for loci evolving under such mutation models the conditional distribution of allele frequency spectrum, given a number of alleles (k), is no longer independent of q (product of effective population size, N_e , and mutation rate, m). In fact, with increasing value of q , the distribution of sample homozygosity (F) shifts towards left (i.e., smaller values). In this investigation, we show that this is also true for microsatellite loci that are closely linked (no recombination) to a locus under additive directional selection. Compared with the case of neutrality, additive directional selection produces an excess of F for a given k . But when two-locus haplotypes are considered as distinct alleles, given the number of segregating haplotypes in a sample (k), the distribution of sample homozygosity (F) is independent of q and consequently the homozygosity test is robust against recombination. (Research supported by US PHS grants CA 75432 and GM 58545 from the US National Institutes of Health).

Characterization of genetic variants within the 10 kb mannose binding lectin (MBL2) locus. *T. Bernig, C. Foster, M. Yeager, J.G. Taylor, S. Chanock.* Pediatric Oncology Branch, National Cancer Institute-ATC, Gaithersburg, MD.

Mannose-binding lectin, MBL2, constitutes an important component of innate immune response. MBL2 is an opsonin that binds carbohydrate structures on microorganisms and activates the complement pathway. MBL2 variants have been associated with disorders characterized by poor immune complex clearance (e.g., polyarthritis), chronic infections (e.g., filariasis) and complications of immunodeficiency states. Three non-synonymous SNPs in exon1 (known as B, C and D) have been shown to decrease oligomerization and complement activation. B and C have arisen independently (B in Eurasian and Native Americans and C in Sub-Saharan Africa) and the cumulative heterozygosity in populations with substantial admixture is between 20 and 35%. Three low efficiency promoter SNPs, (H/L, X/Y and P/Q at positions 550, -221 and +4 respectively) have been described and are in linkage disequilibrium with the structural SNPs, B, C and D. Haplotypes based upon these 6 SNPs correlate with low circulating levels and decreased function. In an effort to comprehensively characterize variation across this locus, we have re-sequenced the 10 kb gene in 32 subjects (3,004 bp of 5' UTR and 675 3' UTR) representing four separate populations. Sixty-five variants including 2 common intronic deletions and 15 novel promoter SNPs have been validated; an estimate of nucleotide diversity by Tajimas test in MBL2 suggests it is not under positive selection ($p=16.9 \times 10^{-4}$ and $q=13.6 \times 10^{-4}$). The proportion of synonymous substitutions/synonymous site (DS) and non-synonymous substitutions/nonsynonymous site (DN) was determined for human MBL2 and the mouse ortholog, *mb1c*, was calculated for the coding region; the value of DN/DS was 0.386, as predicted for a gene not under positive selection. A high density of common SNPs together with the calculated DN/DS suggests purifying selection for MBL2. Furthermore, based upon the high density of newly validated SNPs, further analysis is required to validate the newly determined haplotypes estimated thus far (by E-M), before these data can be applied to investigate MBL2 variants in disease association studies and the study of population genetics.

CD36 as a candidate gene predisposing to severe malaria: polymorphism screening and association with cerebral malaria. K. Omi¹, J. Ohashi¹, J. Patarapotikul², H. Hananantachai², I. Naka¹, S. Looareesuwan², K. Tokunaga¹. 1) Human Genetics, University of Tokyo, Tokyo, Japan; 2) Tropical Medicine, Mahidol University, Bangkok, Thailand.

Plasmodium falciparum malaria remains a major cause of morbidity and mortality in tropical countries. To date, a large number of association studies have highlighted the potential importance of host genetic polymorphisms in the pathogenesis of severe malaria. The human *CD36* gene represents one of most attractive candidate genes for susceptibility to severe forms of malaria. Therefore we screened the all 15 exons, including parts of the neighboring introns, as well as the promoter region of *CD36* and examined a possible association of identified polymorphisms with the severity of malaria in 475 Thai malaria patients. In the screening, a total of 10 polymorphisms were detected, including one novel non-synonymous substitution (S127L). Among these polymorphisms, the -53G>T promoter polymorphism was associated with the protection from cerebral malaria (OR = 0.67, 95%CI = 0.46-0.98, P = 0.043). To examine the influence of this polymorphism on *CD36* transcription level, *CD36* mRNA in monocytes derived from healthy Japanese was semi-quantitatively analyzed by real-time RT-PCR. Our result showed the tendency for mRNA level of *CD36* to decrease in the -53T allele carriers. Mean mRNA level of *CD36* was decreased in the -53T allele carriers compared to the non-carriers by 0.14-fold, although no statistical significant difference was observed. These findings would contribute to understand the mechanism of transcriptional regulation of *CD36* and the pathogenesis of cerebral malaria.

Association Study of Nonsyndromic Cleft Lip With or Without Cleft Palate with AHR, ARNT, and CYP1A1

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The etiology of nonsyndromic cleft lip with or without cleft palate (NSCL/P) is still controversial, but is considered to involve both genetic and environmental factors. One of the suspected environmental factors is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) contained in tobacco smoke, because TCDD causes a high percentage of cleft palate in fetuses when administered during organogenesis in mice. Aryl hydrocarbon receptor (AHR), aryl hydrocarbon receptor nuclear translocator (ARNT), and cytochrome P450 1A1 (CYP1A1) are the enzymes involved in TCDD metabolism, thereby candidate genes for NSCL/P. We assessed whether there is any association of NSCL/P and single nucleotide polymorphisms (SNPs) in the AHR (R554L), ARNT (V623V, P706L), CYP1A1 (I462L) genes using transmission disequilibrium test (TDT). Genomic DNA was prepared from blood samples from 100 Japanese families (287 members) with NSCL/P. Genotyping was performed with a unique allele-specific TaqMan polymerase chain reaction method (Fujii et al., Hum. Mutat. 15:189-196,2000). TDT was carried out by GENEHUNTER 2. Significance levels of AHR (R554L), ARNT (V623V), and CYP1A1 (I462L) were, 0.8312 ($c^2=0.05$), 0.3173 ($c^2=1.00$), and 0.9013 ($c^2=0.02$) respectively. Analysis of ARNT (P706L) could not be performed because of low allelic frequencies. In conclusion the SNPs of AHR, ARNT, and CYP1A1 examined in this study were not associated with NSCL/P in Japanese.

Allele frequency distributions of two VNTR markers in North Indian population and Apo B polymorphism in patients with gall stone disease. *M. Mukherjee, A. Srivastava, A. Kesari, M.K. Singh, G. Choudhury, B. Mittal.*
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Markers like VNTRs at locus D1S80 and Apolipoprotein B 3 HVR are widely used for polymorphic studies in different populations. The polymorphism data in North Indian population at these VNTR loci and association of Apo B polymorphism with gall stone disease is unknown. In this study, we analyzed the polymorphisms of VNTRs D1S80 and ApoB in 86 unrelated normal individuals and carried out the Apo B polymorphism in 36 patients with gall stone disease. Genomic DNA was extracted from blood samples, related regions were PCR amplified and allele sizes were determined. In 86 individuals, 24 and 17 alleles of D1S80 and Apo B respectively determined the presence of 50 and 51 genotypes. By χ^2 analysis, the allele and genotype frequencies for both VNTRs were in Hardy-Weinberg equilibrium. The most frequent allele of D1S80 and Apo B was ~450 bp (28 repeats) and ~600 bp (40 repeats) with frequencies of 0.17 and 0.087 respectively. The Apo B alleles containing 39, 41 and 42 repeats were reported to occur more often in patients in contrast to controls having 40, 42, 43 and 48 repeats. No significant difference ($P > 0.05$) was observed in the allele or genotype frequencies between the patients and controls. The observed heterozygosity for D1S80 was 56 % and Apo B was 53.5 and 50 % in normals and patients respectively. The Apo B polymorphism may not be significantly associated with gall stone disease and therefore cannot be used as the only marker for increased risk of gall stone disease.

Assessment of SNP Haplotype-Block and Estimation of Individual Diplotype in Japanese towards Searching for Disease Susceptibility Gene. *H. Morisaki¹, A. Takashima¹, K. Yamamoto¹, Y. Kitamura², N. Kamatani², T. Morisaki^{1,3}*. 1) Dept. Bioscience, NCVC Research Institute, Suita, Osaka, Japan; 2) Institute of Rheumatology, Tokyo Women's Medical University, Tokyo, Japan; 3) Dept. Molecular Pathophysiology, Osaka University Graduate School of Pharmaceutical Sciences, Osaka, Japan.

Recent studies indicate that patterns of linkage disequilibrium in the human genome are not uniformly distributed. Therefore, the recombination events are thought to have been frequently occurred in the short hot-spots, resulting in formation of low-recombination blocks that separate longer stretches of DNA. Markers within these low-recombination blocks show increased levels of linkage disequilibrium and very low haplotype diversity. This could simplify study of the genetic basis of complex diseases in which causal variants are thought to be common. Here, we have assessed haplotype blocks of several candidate gene loci including APM1, UCP2 and SMTN in Japanese to facilitate to search for the disease-susceptibility gene of the common diseases. We have tried to select marker SNPs to minimize the number of representative single nucleotide polymorphisms (SNPs) required to account for most of the common haplotypes in each block, based on the results of analysis by the LD Support software. By this study, we have defined three haplotype blocks in APM1 locus, one block in UCP2 locus and five blocks in SMTN locus, and estimated individual diplotypes in these loci.

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Novel Coding Variation at codon 351 of *ADH1C* Common in Native Americans. *M.V. Osier, A.J. Pakstis, J.R. Kidd, K.K. Kidd.* Dept Genetics, Yale U, New Haven, CT.

A new *ADH1C* coding variant, Pro351Thr, has been identified and is nearly specific to Native American populations. The newly identified *ADH1C*351Thr* allele is found in most Native American populations we have studied with allele frequencies as high as 26%; only two instances of this allele have been seen in several hundred samples of Eurasian populations. This variant is only 6bp away from the commonly studied Ile349Val polymorphism and likely arose on a haplotype with the ancestral *ADH1C*349Ile* allele. Most of the haplotypes that are common globally can be accounted for by a simple tree of mutations starting from the ancestral haplotype, the one with the ancestral alleles at all sites. From that ancestral haplotype two independent haplotype lineages have evolved accumulating different variants. One lineage involves two identified mutations to generate a haplotype now common in Europe and seen in most populations around the world. A subsequent mutation on this haplotype produced the *ADH1C*351Thr* allele observed in the Americas. The *ADH1C*349Val* allele appears to have arisen on a haplotype from a separate lineage involving two different known mutations. Most significantly, we observe all four possible haplotype combinations of alleles at this new site with alleles of the adjacent *ADH1C* Ile349Val site among Native Americans, presumably due to recombination and subsequent drift, that will translate to four different amino acid sequences of ADH1C. The changes in charge, size, and rotational mobility caused by this new amino acid substitution should be significant. Because four different enzyme forms exist in Native Americans, the kinetics of these enzymes should be studied and haplotypes must be considered in studies of the role of this gene in the metabolism of ethanol and protection against alcoholism in Native Americans. It is also crucial that other previously under-examined populations be screened systematically for more functional variants of the ADH genes. [Funded in part by NIAAA grant AA09379].

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Non-neutral evolution revealed by comparison of gene-based DNA sequence diversity in humans and chimpanzees. *J.A. Schneider, A.E. Anastasio, Y. Morozov, T.S. Kalbfleisch, C.D. Brain, J.L. Vanden Eng, M.S. Pungliya, R. Jiang, C. Xu, R.S. Judson, J.C. Stephens.* Genaissance Pharmaceuticals, Five Science Park, New Haven, CT 06511.

We discovered 3,899 SNPs in 313 human genes by sequencing specific gene regions including exons, exon-intron boundaries, untranslated regions and 5' and 3' flanking regions. In addition to analyzing a group of 82 unrelated humans comprised of African-Americans, Asians, European-Americans and Hispanic Latinos; we also sequenced a single chimpanzee. For 2,968 of the 3,899 human SNPs discovered, we inferred the ancestral state of these polymorphisms from the chimpanzee sequence. For the majority of human SNPs, the chimpanzee sequence contained the common human allele. However, we observed a significant excess of SNPs with minor allele frequencies <20% in which the minor human allele matched the corresponding chimpanzee allele, suggesting that the major human allele at each of these sites is a derived mutation in humans. While positive directional selection is a likely mechanism to explain this observed excess of high frequency, derived alleles, we did not detect a strong overall signal of positive directional selection in these 313 genes using standard analyses of polymorphism and divergence. These results highlight the difficulty involved in understanding the potentially complicated effects of natural selection throughout human evolution.

Relative heterozygosity contributed by alleles of different frequency class is not invariant at microsatellite loci. A. Renwick¹, H. Xu², Y.-X. Fu², M. Kimmel¹, R. Chakraborty³. 1) Dept Statistics, Rice Univ, Houston, TX; 2) Human Genetics Center, Univ. Texas School of Public Health, Houston, TX; 3) Center for Genome Information, Department of Environmental Health, Univ. Cincinnati, Cincinnati, OH.

Allele frequency distribution has played an important role in characterizing the genetic variation. When alleles are divided into different frequency classes and their relative contributions to sample heterozygosity are computed, previous research showed that the relative heterozygosity contributed by alleles of different gene frequency classes is an invariant for neutral alleles under the infinite allele model. This has been used as a neutrality test and it is claimed to be robust against population substructure. In this investigation, we show that under the infinite allele model, the result is only true when q (the product of effective population size, N_e , and mutation rate, m) is small (say, $q \ll 1.0$). For microsatellite loci evolving via a generalized stepwise mutation model, the relative heterozygosity is invariant only for small q (say, $q \ll 1.0$). Since for most microsatellite loci, the mutation rate is relatively high, and consequently, q is greater than 1.0, the test can not be uncritically used to analyze data on microsatellite loci. Applications of this theory to data on microsatellite loci around the human HLA gene region suggest that either this test of selective neutrality has a low power of detecting signatures of natural selection, or strength of selection applicable to the microsatellite loci linked to the HLA gene region is too small to be detected in random sample of individuals from a population. (Research supported by US PHS grants CA 75432 and GM 58545 from the US National Institutes of Health).

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Mitochondrial DNA variation in a Mestizo population from the Southwest Pacific Mexican coasts. *L. Sandoval, C.A. Robles, M.T. Magaña, B. Ibarra, M.P. Gallegos-Arreola, F. Rivas, L.E. Figuera.* Divisiones de Genética, Inmunología y Medicina Molecular, CIBO, IMSS, Guadalajara, Mexico.

Mestizo populations in Mexico have Native American, European (Spanish) and African roots. The admixture took place predominantly through Caucasian and African males with Amerindian females. Genetic markers with blood groups, HLA, and other polymorphisms of nuclear DNA (Lisker et al. 1996, Gorodezky et al. 2001, Cerda Flores et al. 2002) showed that African genetic component varies by geographic region in Mexico. In this report, five mtDNA polymorphisms typed by PCR-RFLP, in 114 Mestizos from the Oaxaca and Guerrero coast communities, are presented. Out of the total samples, 105 (92.1%) Native American and five (4.4%) African haplotypes were found. Besides studies with autosomal polymorphisms (Magaña et al. 2002), present data stress the relevance of African maternal origin in Mexico, with variable marker frequencies from zero (Sandoval et al. 1998) to 4.5% (Green et al. 2000) in both Native and Mestizo populations. This study will contribute to knowledge the African maternal component in Mexican Mestizo populations.

Evidence for genetic susceptibility for Pseudotumor Cerebri in Turkish population. *C.F. Dogulu¹, T. Kansu², V. Baxendale¹, S-M. Wu¹, M. Ozguc², W-Y. Chan¹, O.M. Rennert¹.* 1) Lab of Clinical Genomics, NICHD, NIH, Bethesda, MD; 2) Hacettepe University, Ankara, TURKEY.

Pseudotumor cerebri (PTC) is characterized by symptoms and signs of isolated intracranial hypertension leading to catastrophic effects on the visual system. The clinical picture is caused by raised cerebrospinal fluid (CSF) pressure but the pathogenesis is not clear with the most widely accepted hypothesis being reduced CSF absorption through the arachnoid villi. Although the majority of cases is nonfamilial, there are reports of familial PTC that raise the possibility of some genetic defect that may become clinically manifested after exposure to a precipitating agent. We hypothesized that PTC is a multifactorial disorder with an underlying genetic thrombotic risk factor that predisposes the patients to local thrombus lining arachnoid villi that leads to increased intracranial pressure when the patient is exposed the factors known to precipitate PTC. Exon 10 of factor V was scanned for sequence variation by nucleotide sequencing in 51 PTC patients and 69 controls. Seven patients with PTC were (14%) heterozygous for factor V Leiden (FVL) mutation, versus 11 of 69 control subjects (16%; no significant difference). We also identified a heterozygous single nucleotide substitution (G to A) at nucleotide position 1628 that predicts an amino acid replacement of Arg(R)485Lys(K) in 10 of PTC patients (19.6%), versus 5 of 69 controls (7.2%). Our data revealed: 1) The prevalence of the R485K polymorphism in PTC patients was significantly different compared to the controls by chi-square test ($p < 0.05$) (OR=3.1, 95% CI -0.08 to 24.7%) and might be an important genetic modifier for the development of PTC. Our study is the first to report that a genetic polymorphism is related to an increased risk to PTC. 2) The high frequency of FVL mutation and R485K polymorphism suggests that the present day Turkish population is a perfect admixture of ancient Anatolians and Altaic-speaking, nomadic population originating in Mongolia that migrated to Anatolia. This is in contrast to previous report that ancient Turks contributed little to the gene pool of preexisting Anatolian populations.

Variation in FABP2 5'-Flanking Region Alters Transcriptional Activity and is Associated with Body Composition and Plasma Cholesterol. *C.M. Damcott¹, E. Feingold¹, S.P. Moffett¹, J.A. Marshall², R.H. Hamman², R.E. Ferrell¹.* 1) Human Genetics, Univ of Pittsburgh, Pittsburgh, PA; 2) Univ of Colorado School of Med, Denver, CO.

The fatty acid binding proteins (FABPs) are a family of cytoplasmic proteins involved in intracellular fatty acid transport and metabolism. FABP2, the intestinal-type FABP, is expressed exclusively in enterocytes in the small intestine. Previously, an Ala55Thr substitution was identified in exon 2 of FABP2. *In vitro* studies in Caco-2 cells showed that the Thr-encoding allele is associated with increased binding affinity and transport of long-chain fatty acids and increased secretion of triglycerides. In population studies, the Thr-encoding allele has been associated with increased lipid oxidation, elevated plasma lipids, and impaired insulin sensitivity. We screened roughly 1 kb 5' of the FABP2 initiation codon by direct sequencing and identified three insertion/deletion polymorphisms and three point mutations which were in perfect linkage disequilibrium (FABP2p-ID). We tested the hypothesis that this variation alters gene expression by inserting 5'-flanking sequence of individuals with opposite FABP2p-ID haplotypes into pGL3-basic reporter vector. The constructs were cloned and transfected into Caco-2 cells. Subsequent luciferase assays showed a statistically significant two-fold increase in gene expression of the pGL3-insertion construct over the pGL3-deletion construct ($p < 0.001$). We also tested for association with measures of body composition, plasma lipids, and insulin sensitivity in a sample of non-Hispanic white subjects from the San Luis Valley Diabetes Study ($n=417$). FABP2p-ID was significantly associated with fat mass from DEXA ($p=0.024$), BMI ($p=0.012$), and plasma cholesterol ($p=0.037$) in two-way ANOVAs including sex as a second factor. Plasma free fatty acids ($p=0.602$), plasma triglycerides ($p=0.152$) and HOMA IR ($p=0.165$) were not statistically significant. These results suggest that common genetic variation in the 5' region of the FABP2 gene affects transcriptional activity, presumably leading to alteration in body composition and cholesterol processing.

Dissecting the genetic contribution of a QTL within the DCP1 gene on ACE level. *A. Angius, E. Petretto, I. Persico, M. Fanciulli, D. Bebbere, A. Pernisa, R. Baldinu, G.B. Maestrale, P.M. Melis, M. Pirastu.* Istituto di Genetica delle Popolazioni, CNR, Alghero, SS, Italy.

Circulating ACE level is influenced by a quantitative trait locus (QTL) within the DCP1 gene. In order to clarify the role of this QTL, we carried out haplotype analysis and ACE levels in about 800 individuals (80% of the entire adult population) belonging to a Sardinian genetic isolate (Talana) which exhibits peculiar features such as low number of founders, high inbreeding and 16th generation genealogical records. We determined the serum ACE activity and genotyped 8 SNPs within the DCP1 gene, numbered as follows: (1)A2400T, (2)T2547C, (3)T8128C, (4)13506AluI/D, (5)G14521A, (6)A22982G, (7)23945(CT)3/2, (8)C24599T. These SNPs were chosen based on previous studies which demonstrated their association with ACE level. In addition we studied two new 5' DCP1 gene SNPs: ext1 A/G -37kb, ext2 A/G -75 kb. Linkage disequilibrium (LD) distribution was uneven in the region analysed: two recombination hot spots were detected between ext1 and SNP1 and between SNP2 and 4. The highest LD ($D > 0.9$) was observed between SNPs 4, 5, 6, 7 and between 1, 5. Single SNP association analysis suggest correlation of all the SNPs within the DCP1 gene with ACE mean activity. By haplotype analysis we refine the region of interest from SNP 4 to 7, where we found two common haplotypes DGG2 (50%) and IAA3 (40%), already described in different ethnic groups. ACE levels were normally distributed and showed significant ($P < 0.05$) correlation with age and sex. We detected a mean value of 26.1 U/l for ACE level in homozygotes IAA3, 43.5 U/l in homozygotes DGG2 and 34.1 U/l in heterozygotes IAA3/DGG2. Recombination and gene conversion of these two common haplotypes produce several rare ones differing either for a single or several SNPs included in small LD blocks. ACE phenotype was variously associated with different combination of the SNPs in these haplotypes. Our results suggest that differences in LD blocks arrangements account for the observed variation in ACE activity suggesting that these regions could contain important functional variants.

Analysis of founder effect in *SFTPB* 1549C@GAA (121ins2) mutation. *M. Bahuau*¹, *M. Stuhmann*², *J. Christodoulou*³, *T. Lorant*¹, *C. Houdayer*¹, *J. Elion*¹, *R. Couderc*¹, *M. Griese*⁴, *M. Tredano*¹. 1) Biochimie Biologie Moléculaire, Hôpital Trousseau (MB, TL, CH, RC, MT); Biochimie Génétique, Hôpital Robert-Debré (JE), AP-HP, Paris, France; 2) Institut für Humangenetik, Medizinische Hochschule, Hannover, FRG; 3) Departments of Paediatrics, University of Sydney, Australia; 4) Ludwig-Maximilians Universität, München, FRG.

Surfactant protein B (SP-B) deficiency is an uncommon cause of unexplained respiratory distress in the neonate, *i.e.*, when hyaline membrane disease, pneumonia due to atypical micro-organisms, or congenital heart disease have been ruled out. When complete, SP-B deficiency entails the absence of production of lamellar bodies and tubular myelin (the organized precursors of active surfactant), and eventual storage of protein material within the alveolar space. The onset of disease is congenital and the family pattern is consistent with an autosomal recessive which has been linked to mutations of the SP-B-encoding gene (*SFTPB*). A common mutation (1549C@GAA, or 121ins2) accounts for a sizeable fraction of the mutated chromosomes, although wide allelic heterogeneity has been lately recognized. Since no obvious unstable genetic structure seemed to underlie this lesion (mutation hot spot), we sought to examine the possibility of founder effect. For this purpose, we ascertained the genotype at 8 intragenic SNPs (including 4 novel) in 17 independent 121ins2 chromosomes from 10 probands, parental non-121ins2 chromosomes serving as controls. All subjects were of Caucasian descent (French, Dutch, German, or Australian). One major haplotype was shared by 10/17 121ins2 chromosomes versus 5, or 6/23 control chromosomes (proband and parents from one kindred were heterozygous at one SNP) ($p \gg 0.019$, or $p \gg 0.039$, Fisher's exact test). Overall, these data suggest founder effect with a major haplotype harboring the 121ins2 mutation in the Caucasian population although at least 5 independent haplotypes were elicited. Whether this diversity is due to recurrence of the mutation or intragenic recombination/genetic drift from an ancestral, mutationharboring, haplotype remains to be determined.

MEFV MUTATION SPECTRUM IN FMF PATIENTS FROM KARABAKH. *C. Cazeneuve¹, K. Atayan², D. Genevieve¹, H. Ajrapetyan³, J. Feingold⁴, T. Sarkisian³, S. Amselem¹.* 1) INSERM 468, Hosp Henri Mondor, Creteil Cedex, France; 2) Karabakh; 3) Department of Genetics, Yerevan, Armenia; 4) INSERM 155, Jussieu, Paris, France.

Familial Mediterranean fever (FMF) is an autosomal recessive disorder common in populations of Armenian, Turkish, Arab or Sephardic Jewish ancestry. To delineate the mutation spectrum in another population of Mediterranean extraction, we have investigated a cohort of 50 patients living in Karabakh. As the population from Karabakh has been isolated from the actual frontier of Armenia since the beginning of the XXth century, the spectrum of MEFV gene mutations in this area may differ from that described among Armenian patients. In particular, very rare mutations, absent from the Armenian population samples previously investigated, might be over-represented in this isolated population, due to a founder effect. Screening for mutations of all MEFV coding exons and their intronic boundaries by DGGE revealed a mutation spectrum significantly different from that reported in Armenia. The frequency of non-identified (NI) MEFV alleles is much higher among patients from Karabakh than among those from Armenia (26% vs 7%, $P=5.10^{-5}$). As no particular MEFV haplotype was over represented among the NI alleles, we excluded the existence of a unique and rare MEFV mutation resulting from a founder effect that could have escaped our mutation screening procedure. The spectrum of the identified mutations among Karabakhtsis and Armenian patients is also different: the M694V and R761H mutations were more frequent among Karabakhtsis patients ($P=0.01$ and 0.04 , respectively) and, conversely, the V726A mutation represented only 5% of the identified alleles among Karabakhtsis patients, whereas it accounted for 26% of the characterized FMF alleles in Armenian patients ($P=0.0003$); the M680I mutation was found at a similar frequency in the two populations. No new MEFV mutation was identified among the Karabakhtsis patients. Altogether, these results show that the mutation spectrum differs significantly between the two populations, an observation which may result from the relative independent evolution of each population since the beginning of the XXth century.

Low frequency of Friedreich ataxia in the Mexican Mestizo population. *M. Gomez*¹, *S. Nath*², *S. Bhatti*¹, *A. Rasmussen*³, *S.I. Bidichandani*¹. 1) Biochemistry & Mol Biol, Oklahoma Univ Health Sci Ctr, Oklahoma City, OK; 2) OMRF, Oklahoma Univ Health Sci Ctr, Oklahoma City, OK; 3) National Institute of Neurology, Mexico City, Mexico.

Friedreich ataxia is caused by an expansion of a GAA triplet-repeat sequence in the first intron of the *FRDA* gene. This sequence is polymorphic; short normal (SN) and long normal (LN) alleles have 5-11 and 12-60 triplets, respectively. Disease-causing expanded alleles (E) contain 100-1700 triplets. Most LN and E alleles share the same haplotype at the *FRDA* locus (A-T-C-C at FAD1-ITR4-ITR3-CS2 SNPs) indicating that E alleles arise from LN alleles. LN alleles with >33 triplets have been observed to expand to E alleles upon parental transmission. The allelic frequency of LN alleles predicts the incidence of Friedreich ataxia in most populations. In the Indo-European population 15% of all chromosomes have LN alleles and Friedreich ataxia is the most common inherited ataxia accounting for over 50% of all recessive and sporadic ataxias. Friedreich ataxia is non-existent in South-East Asia and in Native Americans where LN alleles account for <1% of all chromosomes. We have found that the frequency of Friedreich ataxia and LN alleles in the Mexican Mestizo population is intermediate between the Indo-European and Asian populations. Out of 91 recessive and sporadic ataxia patients we found only 9 patients (9.9%) homozygous for E alleles, consistent with the molecular diagnosis of Friedreich ataxia. Only 8 LN alleles were detected among 172 Mexican chromosomes (4.5%). Given the history of the peopling of the Americas we reasoned that the intermediate frequency among the Mestizo population might be due to genetic admixture of European *FRDA* genes in the Native population(s). We have analyzed 344 chromosomes from the Nahua (Aztec) population of Mexico and found that LN alleles account for only 2% of all alleles. In support of our hypothesis, we have found that all E alleles in the Mestizo Friedreich ataxia patients share the same conserved A-T-C-C haplotype of European *FRDA* alleles. These data have important implications for the institution of efficient diagnostic testing in ataxia patients of Mexican origin.

Markers That Discriminate Between European And African Ancestry Show Limited African Diversity. H.E.

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Markers informative for ancestry are necessary for admixture mapping and improving case-control association analyses. For studies in African Americans (AA) this will require markers that can be used to determine if a given genomic region is of European or African ancestry. We show that despite studies indicating high intra-African sequence diversity, markers with large inter-ethnic differences have only small variations in allele distribution among divergent African populations. 32 unlinked diallelic insertion/deletion markers with large frequency differences between one African population and European Americans (EA) were individually genotyped in >150 DNA samples each from Africans in Nigeria and Zimbabwe, EAs, and AAs. Allele frequencies in Nigerians were nearly identical to those in Zimbabweans and dramatically different from those in EAs. Perhaps more surprising, there was a strong correlation between allele distributions in the above African groups and a pooled DNA sample from 21 Pygmies. The variation for the intra-African comparisons, Nigerian/Zimbabwe (mean Wahlund variance $f=0.005$) and Nigerian/Pygmy ($f=0.02$), was very small compared to inter-ethnic African/EA comparisons: Nigerian/EA ($f=0.38$) and Zimbabwe/EA ($f=0.37$). We also examined individual admixture using the Structure program. Africans were clearly separated from EAs without prior knowledge of population affiliation, and no distinction was evident between Nigerians and Zimbabweans. AA showed considerable variation in the proportions contributed from the two parental populations. These studies emphasize the importance of controlling for individual admixture ratios in admixture mapping studies, and provide evidence that the African contribution to AA subjects can be accurately analyzed with a set of obtainable markers that distinguish between European and African ancestry.

The V281L mutation in the *CYP21* gene is a founder mutation that is prevalent among individuals of Ashkenazi Jewish and European ancestry. L. Edelman, R. Kornreich, R.J. Desnick, G.A. Diaz. Dept Human Gen, Box 1497, Mt Sinai Sch Medicine, New York, NY.

Congenital Adrenal Hyperplasia (CAH) results from mutations in the *CYP21* gene, which is located in the MHC region on chromosome 6p21. A duplicated but non-functional copy of the gene is present approximately 50 kb distal to the gene, and a number of mutations have arisen as a consequence of recombination or gene conversion between *CYP21* and the pseudogene. One such mutation, V281L, has been reported in individuals of Ashkenazi Jewish (AJ) and European ancestry with the nonclassical form of CAH. To determine the frequency of carriers of this mutation in the AJ population, an ARMS assay was designed to specifically target the *CYP21* gene and avoid confounding amplification of the pseudogene. Approximately 1,000 anonymous AJ and 120 anonymous non-Jewish (NJ) individuals of European ancestry were tested by allele specific oligonucleotide hybridization (ASOH) for the presence of the V281L allele. The carrier frequencies of this mutation were 17% and 5.8%, respectively. Sequence analysis revealed that the mutation arose by gene conversion of ≥ 262 bp to ≤ 372 bp, such that the *CYP21* gene, containing the V281L mutation, also had other changes that were present in the pseudogene. Both AJ and NJ carriers possessed this block of changes that spanned intron 6 through exon 7, suggesting a common origin. This finding was in agreement with reports that HLA-B14/DR1 haplotype was in linkage disequilibrium (LD) with the mutation. To determine the extent of LD present at this locus, microsatellite markers flanking the region were tested on 40 AJ V281L, chromosomes, 50 AJ non-carrier chromosomes and seven NJ V281L carriers. The results were consistent with the presence of a small but conserved haplotype surrounding the locus that is less than a Mb in size in both AJ and NJ carriers. These findings confirm the existence of a founder allele for the V281L mutation and raise some interesting questions about the actual age of the allele, the rate of recombination in the region and the potential source of selective pressure on the locus that has resulted in its high frequency in both populations.

Genetic variation near RXRb is associated with increased cholesterol levels in Hispanic males. *S.P. Moffett¹, C.M. Damcott¹, M. Barmada¹, E. Feingold¹, J.A. Marshall², R.F. Hamman², J.M. Norris², R.E. Ferrell¹.* 1) Human Genetics, Univ of Pittsburgh, Pittsburgh, PA; 2) Univ of Colorado School of Medicine, Denver, CO.

The retinoid X receptors (RXRs) are members of the nuclear receptor superfamily that heterodimerize with a number of the other nuclear receptors including the peroxisome proliferator activated receptors (PPARs) to form transcriptionally active complexes. The PPAR:RXR complex acts as a transcription factor, altering gene expression related to lipid metabolism and adipocyte differentiation. RXRb, one of three RXR genes, is expressed ubiquitously in human tissues. The purpose of this analysis was to determine the effect of variation near RXRb on plasma lipid levels in Hispanic individuals enrolled in the San Luis Valley Diabetes Study. An A to T transition 140bp downstream of the last exon (RXRb140) was typed in 247 individuals of Hispanic descent using an *Mbo*I restriction enzyme site. The frequency of the less common T allele was 0.271 and conformed to the expectations of Hardy Weinberg equilibrium. Analysis of variance showed an association between the T allele of RXRb140 and increased total cholesterol levels (AA=193mg/dL, AT=208mg/dL, TT=202mg/dL; $p=0.007$) that accounted for 4% of the trait variation. Plasma triglycerides and fasting free fatty acids showed no statistically significant associations with RXRb140 in this sample ($p=0.177$ and $p=0.394$, respectively). To further explore the association between RXRb140 and cholesterol, the analysis was repeated separately for males and females. The female subjects showed no statistically significant association between RXRb140 and total cholesterol ($p=0.128$), but the male individuals showed an apparently dominant effect of the T allele on total cholesterol levels that explained over 7% of the variation in the trait ($r^2=0.071$). Presence of the T allele increased total cholesterol levels by 18 to 20mg/dL as compared to the AA homozygotes (AA=186mg/dL, AT=204mg/dL, TT=206mg/dL; $p=0.013$). These results suggest a gender specific role for RXRb in cholesterol metabolism, presumably through interaction with other nuclear receptors.

Population variation at human SNPs associated with HIV resistance, susceptibility and therapy. *J.J. Martinson, A.L. Edge, M. Lewis, P.M.R. Aldred.* Inst. of Genetics, University of Nottingham, Nottingham, England.

Several human genetic loci are now known to influence the severity of HIV disease, and it is likely that many more remain to be discovered. Most aspects of HIV disease progression from initial infection through to end-stage AIDS, and even the response to common antiretroviral drugs, have been shown to be affected by host genetic loci. SNP variation at these loci is likely to become increasingly important, in predicting disease outcome and in influencing the choice of antiretroviral therapy, for individuals and also for populations. The full extent of such variation, however, is not clear at present. Here we integrate data obtained from a global survey of variation at seven SNP and other alleles known or suspected to influence HIV outcome, including three (IL-10 C-592A, eotaxin C-1382A, and MDR1 C3435T) not previously studied at the global level.

Our results show substantial variation between populations in these alleles. Many populations have high frequencies of several resistance-associated SNPs, and so their members may carry more than one resistance allele. The observed variation cannot be easily partitioned along lines of geographical proximity or of ethnic group, regardless of how these groups are defined. With the exception of the well-known cline in frequency of the CCR5-D32 allele in Europe, no strong geographical trends are seen at these loci. For example, the frequency of the MDR1 3435C allele is substantially higher in sub-Saharan Africans than in other populations, but the overall SNP allele frequency distribution does not correlate well with geographic proximity ($r=0.011$). This allele is associated with a higher rate of cellular excretion of antiretroviral drugs, and hence their reduced bioavailability; a knowledge of its prevalence in different populations may affect prescribing practice. Our results show that it is not sufficient to make assumptions of an individual's HIV resistance phenotype based on their membership of a particular ethnic group, however. A thorough determination of each individual's genotypes provides a more reliable means of predicting disease progression and response to treatment.

Evidence for the changing incidence of cystic fibrosis. *V. Scotet¹, M.P. Audrezet¹, M. Roussey², G. Rault³, M. Blayau⁴, M. De Braekeleer^{1,5}, C. Ferec¹.* 1) Dept of Molecular Genetics, INSERM EMI 01-15, Brest, France; 2) CHRU Ponchaillou, Rennes, France; 3) Centre de Perharidy, Roscoff, France; 4) Dept of Molecular Genetics, Rennes, France; 5) Dept of Cytogenetics, Brest, France.

The cloning of the gene responsible for cystic fibrosis (CF) in 1989 and the identification of its mutations have enabled to implement efficient strategies of diagnosis and disease prevention, such as neonatal screening and prenatal diagnosis. Taking into account the situation of Brittany, a region of western France where CF is common and where a neonatal screening program was set up 13 years ago, the aim of this study was to assess the changing incidence of CF and to determine how it was influenced by different programs. From 1992 to 2001, the neonatal screening program identified 123 CF children, leading to an incidence of 1/2838. Over the same period, we collected the prenatal diagnoses realised in Brittany in families related to a CF child, as well as those made following the detection of an echogenic bowel during pregnancy. By considering the 54 CF-affected pregnancies terminated during these 10 years, the corrected CF incidence was 1/1972. Prenatal diagnosis was therefore responsible for a global decrease of 30.5% in CF incidence. Two-thirds of these 54 abortions were chosen by couples related to a CF child (n=36); this led to a change of 22.6% in incidence. The 18 other abortions were made after the detection of an echogenic bowel; this ultrasound examination of pregnancies led to a decrease of 12.8% in CF incidence. Moreover, neonatal screening, through early diagnosis, enables couples to benefit from prenatal diagnosis, even before the first symptoms appeared on their previously screened child. This was the case for 13 couples in our study, which modified the incidence 6.1 percent. Finally, cascade screening, through the detection of 17 new 1-in-4-risk couples in the kin of a CF child, led to a decrease of 3.9% in incidence. This study is the first one able to determine the evolution of CF incidence through both neonatal screening, prenatal diagnosis, ultrasound exams and cascade screening. Awaiting the development of a cure, prevention remains an important aspect in the management of CF.

Effect of consanguinity on the incidence of several autosomal recessive disorders in the Azorean island of São Miguel (Portugal). *A. Simas, P.R. Pacheco, C.C. Branco, B.R. Peixoto, L. Mota-Vieira.* Genet and Molec Pathol Unit, Hospital of Divino Espirito Santo, Azores Islands, Portugal.

São Miguel island is the biggest and the most populated island (131,510 inhabitants, Census 2001) of the Azores. The present-day population is the result of <22 generations and it shows evidences of relative homogeneity. Recently, we demonstrated that »50% of the subscribers listed in the 2001 telephone book are distributed by a very small number of surnames (26 out 1,315) and that 2.2% have surnames that appear only once. Here we investigate the role of consanguinity in 20 unrelated patients affected with one of the following autosomal recessive disorders: cystic fibrosis, CF (6), hereditary hemochromatosis, HH (5), spinal muscular atrophy type I, SMA-I (3), hereditary spastic paraplegia, HSP (2), Gilbert syndrome, GS (1), Hurler syndrome, HS (1), Friedrich ataxia, FA (1) and metachromatic leucodystrophy, ML (1). To that end, we surveyed the patient's ascending genealogy up to the 6th generation. We collected maternal and paternal surnames, and place of birth for each relative. All families were classified according to the degree of parental consanguinity: closely related (second cousins or closer, $F^3=0.0156$), suspected consanguinity (same village origin and sharing common surnames) and non-consanguineous (no relationship). Among these families, 8 were consanguineous, 2 had suspected consanguinity and 10 were non-consanguineous. The mean inbreeding coefficient of the 8 patients whose parents were closely related is very high ($F=0.0596$), if compared to the island population ($a=0.00069$). Moreover, they are affected with different pathologies: CF (2), HH (2), SMA-I (2), HSP (1) and FA (1). The geographic distribution of all 20 families shows that 12 come from different rural communities, which is consistent with the fact that »60% of the population lives in rural area. Although the number of families analyzed here is small, the data indicate that the recessive mutated alleles may not be rare. The characterization of the pattern of all recessive diseases in this island is crucial for future studies of genetic epidemiology. (lmv.hospdelgada@mail.telepac.pt).

Use of multilocus DNA fingerprinting for the studies of human populations from Eastern Europe and North Asia. *E.V. Shabrova*¹, *E.K. Khusnutdinova*², *A.I. Mikulich*³, *L.A. Tarskaia*¹, *S.A. Limborska*¹. 1) Dept. of Human Molecular Genetics, Institute of Molecular Genetics, Moscow, Russian Federation; 2) Bashkir Science Center of Ural Branch of Russian Academy of Sciences, Ufa, Russian Federation; 3) Institute of Arts, Ethnography and Folklore, Minsk, Byelorussia.

We have used the technique of DNA fingerprint analysis with M13 phage DNA as a probe to investigate rather heterogeneous group of 14 human populations from Eastern Europe and Asia. These populations belong to three language families: Indo-European language family (Slavonic branch: Russians, Byelorussians), Uralic language family (Finno-Ugric branch: Mari, Mordvinians, Udmurts, Komi), Altai language family (Turkic branch: Bashkirs, Tatars, Chuvashes, Yakuts). As the result of the analysis of restriction fragments patterns generated by hybridization the size matrix of all the fragments from 10,000 bp to 2,000 bp was constructed. The set of individual patterns was presented as a binary matrix like "Object-Trait". Coefficients of differentiation for the linguistic hierarchical structure were estimated according to Lynch (1990. *Mol. Biol. Evol.* 7(5)). Phylogenetic trees were constructed and multidimensional scaling was carried out on the bases of some types of the genetic distances matrices calculated from the populations profiles of fragments frequencies. Multiple correspondence analyses were applied for the treatment of binary populations matrices. Levels of population subdivision in the groups of closely related populations appeared to be quite different. In our study two territorially separated Byelorussian populations produced no regional differences in contrast to four Bashkir populations. On the whole different methods of statistical analysis have demonstrated that the populations of Slavonic branch form the common group isolated from the populations of Volga-Ural region, Yakuts found to be most close to some of Bashkir populations and Komi to be the most distant from others. In our studies we have not found the absolute correlation between the data of multilocus DNA fingerprinting and the linguistic affinity data.

Polymorphism of the MUC1 gene and haplotype analysis in healthy individuals and in patients with gastric and oesophageal diseases. A. Teixeira¹, J. Fowler¹, L. Vinall¹, L. Lovat², M. Sarnier², D. Swallow¹. 1) The Galton Laboratory, Department of Biology, University College London, London, United Kingdom; 2) Department of Gastroenterology, University College London, London, United Kingdom.

Background: The membrane mucin MUC1, like other epithelial mucins, contains a long and variable length array of tandem repeat units. We and others have previously shown that short tandem repeat alleles are associated with an increased risk of developing *H. pylori* gastritis (Vinall et al, *Gastroenterology*, 2002, 123, in press) (Silva et al, *Eur J Hum Genet*, 2001, 9: 548-52). Despite the high level of allelic variability of MUC1, two polymorphic markers flanking the array, one SNP and one microsatellite, are in tight linkage disequilibrium. **Aims:** The aim of this study is to further characterise the polymorphism and haplotype distribution of MUC1 in the patients with gastritis as well as other patient groups and in healthy people of different ancestry. **Methods:** Southern blot analysis of *HinfI* restriction digests was used to determine TR length. Multiplex PCR, restriction enzyme digestion and analysis on ALF express (Pharmacia Biotech) were used for analysis of the flanking markers. MVR analysis (Minisatellite Variant Repeat) was used to generate maps of the tandem repeats. **Results and Discussion:** The flanking, as well as TR, polymorphisms were examined in cohorts of individuals of northern European ancestry including the previous studied patients with gastritis and 83 new patients with oesophageal carcinoma. Haplotype analysis showed some differences in the patient groups: in particular, in the gastritis group there was an over-representation of small alleles of rare haplotype. MUC1 polymorphism was also examined in a cohort of 94 Nigerians. In this population the flanking markers showed the same alleles as those present in Northern Europe, also in strong linkage disequilibrium. MVR analysis of MUC1 in a selection of patients and controls shows evidence of genetically determined tandem repeat sequence variability and the maps could be classified into classes with respect to TR map and haplotype.

Patterns of genetic variation at interleukin-13 (IL13) in human populations. *E. Tarazona, S. Tishkoff.* Dept. of Biology, Univ. of Maryland, College Park, MD.

IL13 is a pro-inflammatory cytokine that plays a critical role in the induction of Th2 immune response against extracellular parasites. It is also shown to be a susceptibility locus for asthma. We have sequenced 2891bp encompassing the entire gene in 150 individuals from 12 ethnic groups from West Africa (Maasai, Hadza), East Africa (Yoruba, Igbo, Fulani), MiddleEast/Europe (Lebanese, Northern European, Russian), China and South America (Quechua, Cayapa, Ashaninka). The nucleotide diversity is 0.0012, corresponding to 23 SNPs. Only one SNP is a non-synonymous substitution (Arg130Gln) in the fourth exon. Western-African populations show the highest level of genetic variability, followed by East-Africa, Europe and South America. African populations have higher level of intra-genic recombination and lower level of linkage disequilibrium (LD) than non-African populations. While LD decreases with increased distance between SNPs in Eurasian and Amerindian populations, African populations show no correlation between genetic distance and LD. We analyzed the spectrum of allelic diversity across the gene using several neutrality tests in order to test the hypothesis of natural selection acting on the Arg130Gln substitution. Fu and Lis test indicates a significant excess of rare SNPs in both African and Amerindian populations in the same two regions of the gene. The excess of rare alleles in African populations is consistent with evidence from numerous loci indicating past population expansion in Africa. However, because South Amerindian populations do not show any evidence of past population expansion based on nuclear loci, and due to significant LD between the Arg130Gln substitution and the region where an excess of rare alleles are observed, positive selection could have influenced the allelic spectrum of IL13 in these populations. Analysis of additional populations is required to confirm this pattern. Our identification of 16 common SNPs and the haplotype structure is informative for design of haplotype association studies for IL13 and common autoimmune disorders such as asthma. Funded by Burroughs Wellcome and Packard Career Awards and NSF grants to SAT.

Selection and gene conversion produce a unique haplotype structure at X-linked color vision genes. *B.C. Verrelli, S.A. Tishkoff.* Dept Biol, Univ Maryland, College Park, MD.

Trichromatic color vision in humans is made possible by three genes that code for red, green, and blue light-sensitive cone pigments. The red and green pigment genes are the result of a relatively recent tandem duplication on the X chromosome and frequently undergo unequal crossing over and gene conversion events, much of which leads to color blindness. Studies have shown that these two genes share many polymorphisms and that mutations in the red pigment gene can cause shifts in the visual color spectrum. Because much of this variation is not associated with color vision deficiencies, it raises the question whether this variation has an adaptive explanation. In this current study we characterize the nucleotide diversity for a 5.5-kb region of the red and green pigment genes to address the molecular and functional variation associated with color vision. Our random sample of over 160 Africans and 60 non-Africans for exons and introns finds more than 75 SNPs in the red pigment gene that account for over 12 amino acid substitutions and 22 protein haplotypes due to linkage equilibrium between some SNPs only 10 bp apart. We also find that protein haplotypes differ in frequency between African and non-African populations. Although gene conversion homogenizes red and green intron sequences, purifying selection apparently maintains several amino acid residues that are responsible for the spectral properties of the two genes. However, our comparisons with chimpanzee sequences indicate that balancing selection also maintains amino acid variation at these genes. This indicates that protein haplotypes, and not single amino acid variants, should be used to define color vision phenotypes. This study indicates that gene duplication and gene conversion may account for blocks of low linkage disequilibrium (LD) that are often found in genomic regions of high LD. It is also apparent that color vision genes rival the level of polymorphism found at other duplicated genes that show high functional diversity, like the HLA loci. Additional studies of this nature may reveal how gene duplication and selection account for novel patterns of genetic diversity and LD in the genome.

Malarial selection and the Dogon: patterns of DNA sequence variation at the b-globin locus. *E.T. Wood¹, Z. Mobasher¹, B. Strassman², M.F. Hammer¹.* 1) Ecology & Evolutionary Biology, University of Arizona, Tucson, AZ; 2) Anthropology, University of Michigan, Ann Arbor, MI.

To better understand the role of selection in shaping patterns of variation at loci that confer resistance to malaria, we sequenced 6-kb from b-globin and Dmd intron 44 (Dmd44) in 32 individuals from the Dogon, a population who reside in an endemic malarial environment in Mali, West Africa. B-globin contains two mutational sites that are known to confer resistance to malaria while Dmd44 is not subject to malarial selection and likely reflects demographic processes. Patterns of variation at these two loci were also compared in a global panel of 41 humans. Levels of pairwise nucleotide diversity at both b-globin and Dmd44 in the Dogon, as well as in the global sample, were very close to the average for the human genome ($\pi = 0.11-0.15\%$). However, there was a much higher proportion of segregating sites at the b-globin locus in the Dogon compared with the global sample ($\theta = 0.22\%$ and 0.14% , respectively). These patterns contrasted with those found at the Dmd44 locus which showed fewer number of segregating sites in the Dogon relative to that found in the global sample ($\theta = 0.09\%$ and 0.15% , respectively). The contrasting patterns of variation at these two loci in the Dogon are reflected in very different frequency distributions of polymorphisms. B-globin exhibited an excess of rare polymorphic sites in the Dogon (Tajimas $D = -0.73$; $0.10 > P > 0.05$); whereas, patterns of variation at Dmd44 in the Dogon showed a slight excess of intermediate frequency polymorphisms (Tajimas $D = 0.72$, $P > 0.10$). These results are consistent with a model of selection because demographic processes such as population expansion and subdivision are expected to affect all loci in a similar manner. To further support this inference and to clarify the model of selection acting at b-globin, these loci are being sequenced in another sub-Saharan African population from a non-malarial area.

A reference distribution of Fst values for biallelic DNA markers. A.J. Pakstis, J.R. Kidd, K.K. Kidd. Dept Genetics, Yale U., New Haven, CT.

Demographic and evolutionary histories shape the expected allele frequency variation among populations, measured as Fst. Random genetic drift (r.g.d.) will account for much of this variability for unlinked loci and even for tightly linked markers if LD is incomplete. To avoid variability in Fst estimates due to using different sets of populations, we studied 94 biallelic markers (mostly SNPs) on the same set of 32 population samples (avg ~53 persons/pop) distributed across major world regions. A distribution of Fst values for many markers based on a *standard* set of geographically diverse human populations can define the variation expected among sites due to r.g.d. Extremely large or small Fst values support some form of selection at work, at least historically. Most of the markers studied are within the intronic regions of 28 loci or gene clusters--23 widely separated regions on 16 autosomes. None of the markers are known to have been subject to selection. Most were prescreened to avoid monomorphism in a majority of populations. So, the reference distribution does not have as many Fst values close to zero as it would if all low frequency polymorphisms were included. The mean of the Fst distribution for 32 populations is .14 (median .13, std dev .07; range .04 to .37). With 2 to 7 SNPs at each of 21 loci (84 biallelic), it is possible to test for similarity of Fst values at the same locus. The eta correlation ratio is .61 for this large subset but it is not significantly different from zero. A trend may exist for some weak global correlation based on tight linkage ranging from 1 to 120 kb. When Fst is computed for the same 94 polymorphisms within 6 continental regions the avg Fst values are much smaller (Africa .06, Europe/SWAsia .03, E.Asia .04, Pacific .06, N.America .05, S.America .08) than for the world as a whole reflecting the resemblance of populations in the same geographic area. Variation of Fst values is greatest for African pops when both the range (.00 to .42) and std dev (.05) are considered but large std devs do occur for Pacific(.07), N.Amer(.05), S.Amer(.07). Europe/SWAsia and E.Asia have the smallest ranges (.00 to .09; .00 to .16) and std devs(.02,.03). Support: NIH GM57672, MH62495, AA09379, NSF BCS-9912028.

No evidence for heterozygote advantage at MTHFR in patients with lumbosacral myelomeningocele or their relatives. *E. Rampersaud¹, C. Brusata¹, K. Metcalf², E. Melvin¹, M.C. Speer¹ and NTD Collaborative Group.* 1) Center for Human Genetics, Duke University, Durham, NC; 2) North Carolina School of Science and Math, Durham, NC.

Folate supplementation appears to reduce the risk for NTDs. Methylenetetrahydrofolate reductase (MTHFR) is a candidate gene in the folate metabolism pathway that has been associated with mothers and children with NTDs in some populations. The C677T mutation in the 5,10-methylenetetrahydrofolate-reductase gene (MTHFR) yields a common thermolabile variant (T) with a reduction of 30-50% activity compared to the allelic form (C). Previously, Weitkamp et al. reported a heterozygote advantage of the MTHFR gene in patients with neural tube defects and their relatives. We examined our own series of 166 American Caucasian families for evidence of heterozygote advantage. Our sample included 166 probands, 162 mothers, 136 fathers, and 120 unaffected siblings. The allele transmission ratio from C/T heterozygous parents was 85.88C: 86.85T to offspring with NTD ($X^2 = 0.005$, $p = 0.82$) and 55.27C: 63.7T to unaffected offspring ($X^2 = 0.600$, $p = .44$). For the number of offspring of each genotype (C/C, C/T, T/T), expectations among the 286 total offspring ($X^2 = 49.18$, $p = 0.56$), the 166 affected offspring ($X^2 = 16.74$, $p = 0.56$), and the 120 unaffected offspring ($X^2 = 9.58$, $p = 0.70$) did not differ from Mendelian expectations. In addition, the number of offspring of each genotype among the 226 offspring of C/T heterozygous parents did not differ from Mendelian expectations ($X^2 = 1.17$, $p = .56$). There is neither an excess of heterozygotes (107.2 observed, 75.16 expected) nor a deficiency of C/C (75.01 observed, 75.16 expected) or T/T homozygotes (43.46 observed, 37.68 expected). There was no departure from Hardy-Weinberg equilibrium in the mothers (65 C/C, 82 C/T, 20 T/T observed; 67 C/C, 80 C/T, 22 T/T expected; $X^2 = 0.71$, $p = .40$), nor in fathers (64 C/C, 85 C/T, 17 T/T observed; 68 C/C, 76 C/T, 21 T/T expected; $X^2 = 2.0$, $p = .16$). Therefore, our data does not demonstrate the existence of any heterozygote advantage.

Genetic and sequence based analysis of the human *TGFB3* in cleft lip and palate and cross-species homology comparisons. *Y. Watanabe*^{1,2}, *E. Park*¹, *S. O'Brien*¹, *C. Nishimura*¹, *B. Ludwig*¹, *J.C. Murray*¹. 1) Dept Pediatrics, Univ Iowa, Iowa City, IA; 2) Dept Pediatrics and Child Health, Kurume Univ, Fukuoka, JAPAN.

Genetic association studies have been increasingly successful identifying mutations within genes that cause human complex traits. Cleft lip and palate is one such complex trait and is a common birth defect with a frequency ranging from 1/500 to 1/2500 depending on the ethnic or geographic origin of the population under study. Previous studies of genetic linkage and association have supported a role for *TGFA*, *MSX*, and *TGFB3* in non-syndromic clefting in humans. A complimentary approach, involving establishment of a mouse knockout for *TGFB3*, also supports a role for *TGFB3* in craniofacial development. In this report, we described a detailed sequence based comparison of the *TGFB3* genomic locus and its surrounding 5 and 3 flanking regions for single nucleotide polymorphisms. A collection of four such markers were then studied in detail on one Caucasian and one Asian based panel of mother/father/infant nuclear triads for evidence of Transmission Disequilibrium Test (TDT) distortion and haplotype analysis. A subset of individuals totaling approximately 180 were also directly sequenced in all coding regions as well as in regions of homology identified by comparing the human and mouse genomic sequences. Finally, a clone for the *Fugu TGFB3* homolog was identified and sequenced in cross-species homologies between *Fugu*, mouse and human. While no specific mutations that are clearly etiologic could be identified in any of the individuals directly sequenced, the statistical support for a role in *TGFB3* remains, and haplotype analysis suggests that a regulatory element located somewhere within the coding structure is the most likely etiologic event.

Study of genetic diversity by surname analysis of the Azorean population (Portugal). *C.C. Branco, L. Mota-Vieira.*
Genet and Molec Pathol Unit, Hospital of Divino Espirito Santo, Azores Islands, Portugal.

Azores is an archipelago located in the North Atlantic Ocean (parallel 38). It is composed by 9 islands dispersed over three groups: oriental (São Miguel and Santa Maria), central (Terceira, Graciosa, Pico, São Jorge and Faial) and occidental (Flores and Corvo). The present-day population stands at 242,073 inhabitants (Census 2001), being the result of <22 generations. About 75% of the population is concentrated on two islands, São Miguel (50%) and Terceira (25%), whereas the remaining 25% are distributed among the other 7 islands.

In order to investigate the genetic structure of the Azorean population we conducted a survey based on frequencies of surnames listed in the 2001 telephone book. We first calculated the following parameters: isonymy (I), coefficient of inbreeding (F_{st}), coefficient of kinship between islands (R_i), abundance of surnames according to Fisher (a), Karlin-McGregor's migration rate (n) and Nei's distance. As expected, the results show that the migrating phenomenon occurs mainly towards the big islands. The values of migration rate range from 0.247, for the smallest island of Corvo, to 0.0027 for the biggest one, São Miguel. The assessment of the isonymy similarity was performed by calculating Nei's distance using the UPGMA method. The dendrogram obtained reveals three major clusters corresponding to the geographic distribution of the nine islands. On the other hand, Graciosa, the second smallest island, presents the lowest value of abundance of surnames ($a=15.75$), suggesting a greater genetic isolation when compared to other islands. Moreover, the diversity analysis of surnames for all islands demonstrates that 11% of all 57,385 subscribers are distributed by only 3 surnames. Finally, the value of F_{st} obtained for this population (0.0039) indicates a little genetic differentiation (Wright's $F_{st}<0.05$). In conclusion, our data provides evidences that the Azorean population is relatively homogeneous, representing an ideal source for genetic epidemiological studies, as well as, for mapping of alleles underlying complex and autosomal recessive disorders. (claudia.branco@clix.pt).

Linkage Disequilibrium's extension in Corsica. Looking for genetic isolates. *V. Latini¹, D. Poddie², G. Vona¹, L. Varesi³, M. Memm³, G. Sole², S. Doratiotto², A. Cao², M.S. Ristaldi².* 1) Biologia Sperimentale, Cagliari University, Monserrato, CA, Italy; 2) INN-CNR, Selargius, CA, Italy; 3) Corte University, Corte, France.

Genetic isolates represent an exceptional resource for the identification of complex disease susceptibility genes. Recent reports have in fact demonstrated that in isolated and conservative populations the extension of the Linkage Disequilibrium (LD), the non-random association of alleles at closely linked loci, is particularly high. The higher the extension of the LD, the lower is the number of polymorphism markers which have to be examined to find an association with a particular disease. This study has been designed to examine the extension of LD on a region of the X chromosome (Xq13.3) in genetic isolates of the island of Corsica. We have analysed the LD extension in three populations of Corsica: Niolo (n = 39), Bozio (n = 35) and Corte (n = 40), which are genetically isolated and conservative compared to the rest of Corsica. Samples (only unrelated males) have been analysed using seven dinucleotide microsatellite markers on chromosome X: DXS983, DXS986, DXS8092, DXS8082, DXS1225, DXS8037 e DXS995. Microsatellite genotyping was performed using ABI377 sequencer and Gene Scan 3.1 and Genotyper 2.0 software. We measured LD using D' statistic (Lewontin 1964). The significance of non-random allelic association between pairs of microsatellite loci was calculated using Fishers exact test. Our preliminary results show a strong degree of LD in all the three Corsicans isolates. The extent and the strength of the LD is higher than that of the general population of Corsica and comparable to that reported for other isolates such as the village of Gavoi (Sardinia) and Saami (North Europe). This data indicated that the populations investigated in the present study are well suited for associations studies aimed at the mapping of genes involved in the pathogenesis of complex diseases. Nevertheless, since the D' value is influenced by the sample size we are currently expanding our sample to validate these results.

Genetic history of linguistically diverse Tanzanian populations inferred from mtDNA. *H.M. Mortensen¹, M.K. Gonder¹, J.B. Hirbo¹, B.C. Verrelli¹, J.L. Mountain², S.A. Tishkoff¹.* 1) Dept of Biology, Univ Maryland, College Park, MD; 2) Dept. of Anthro. Sciences, Stanford Univ, Stanford, CA.

East Africa is a key region to sample genetic diversity because it is a likely site for the origin of all modern humans and because there are many linguistically diverse ethnic groups living in close proximity to one another. Tanzania is one of the most ethnolinguistically diverse regions of Africa, with populations speaking languages belonging to the four, major linguistic families of Africa: Khoisan, Afro-Asiatic, Nilo-Saharan and Niger-Kordofanian. The genetic history of these populations remains largely unknown. Analysis of genetic variation in Tanzanian populations enables us to estimate long-term effective population sizes, timing and extent of historic population expansions or contractions, levels of exchange between populations, and correlations between genetic and linguistic variation. A linguistically and culturally diverse panel of >400 Tanzanians representing the four major linguistic families of Africa and practicing diverse subsistence methods (pastoralism, agriculture, and hunting/gathering) was analyzed for mitochondrial DNA variation. These include Khoisan-speaking Hadza and Sandawe, Nilotic-speaking Maasai and Datog, Cushitic-speaking Burunge and Iraqw, and Bantu-speaking Gogo and Turu. Control region (CR) sequence encompassing both hyper-variable segment I (HVSI) and II (HVSII) was generated. Phylogenetic and population genetic methods were applied to analyze these data, in comparison to a broad African and global human dataset. Genetic and linguistic affinity did not correspond in all instances. The Tanzanian panel was generally found to exhibit high genetic diversity, which is in concordance with the long and complex demographic history of this geographic region. We observe the genetic signature of a number of recent migration events into northern Tanzania. These data help clarify existing linguistic and archaeological interpretations regarding East African population history as well as early migration of modern humans out of Africa. Funded by BWF and Packard career awards, Wenner Gren, Leakey, and NSF grant BCS-9905396 to ST.

Assessing population structure and its effects on association studies in a genome wide SNP dataset. *J.L. Marchini¹, P. Donnelly¹, M. Boyce-Jacino², L. Cardon³*. 1) Department of Statistics, Oxford University, Oxford, UK; 2) Orchid BioSciences, Inc; 3) Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Oxford OX3 7BN, UK.

Association studies using genome wide SNP datasets have been suggested as a powerful method to detect the genetic basis of common complex diseases. A potential problem with such studies is the presence of undetected population structure in the sample of cases and controls. This raises two general issues: (i) how much underlying stratification is there in various human populations, and when might this pose problems for association studies, and (ii) are the suggested approaches for correcting for population structure in case-control studies accurate and efficient.

We investigate these question across and within populations in the context of a genome wide dataset in which around 12,000 SNP's have been genotyped in 130 individuals from 3 populations. We assess the ability of model based methods (like STRUCTURE) to detect fine levels of differentiation, such as that between Asian-Americans of Japanese/Chinese origin and the performance of both Structured Association and Genomic Control approaches to association studies in this context.

Genetic Epidemiology of Severe, Early-Onset Chronic Obstructive Pulmonary Disease: Risk to Relatives for Sensitive Spirometric Phenotypes. *D.L. DeMeo^{1,2}, S.T. Weiss^{1,2}, J.M. Drazen², H.A. Chapman², J.J. Reilly², L.C. Ginns³, F.E. Speizer^{1,2}, E.K. Silverman^{1,2}.* 1) Channing Laboratory, Boston, MA; 2) Brigham & Women's Hospital, Boston, MA; 3) Massachusetts General Hospital, Boston, MA.

The Boston Early-Onset COPD study has enrolled family members of probands identified with severe, early-onset COPD before age 53, and forced expiratory volume in one second (FEV1) less than 40 percent predicted in the absence of alpha 1-antitrypsin deficiency. Current or ex-smoking, first degree relatives of early-onset COPD probands previously have been demonstrated to have significantly lower FEV1 and FEV1/FVC values than control subjects, suggesting that genetic risk factors for COPD are expressed in response to cigarette smoking. We hypothesized that first-degree relatives of early-onset COPD probands would also demonstrate alterations in more sensitive indicators of pulmonary function, such as the ratio of forced expiratory flow in the mid-portion of forced vital capacity divided by forced vital capacity (FEF25-75/FVC). This measure has been suggested to represent dysanaptic lung growth, a disproportionate growth between airways and lung parenchyma. Using generalized estimating equations, FEF25-75 and FEF25-75/FVC were analyzed in 348 first-degree relatives of probands and 83 sex and smoking matched controls. A statistically significant risk to first-degree relatives of individuals with early-onset COPD was demonstrated in ever smokers for abnormalities in FEF25-75 ($p=0.013$ women, $P=0.021$ men) and FEF25-75/FVC ($p=0.008$ women, $p=0.002$ men), but not FVC alone ($p=0.185$ women, $p=0.706$ men). No significant differences were found among lifelong never smokers. Because of the cross-sectional nature of these findings we are unable to distinguish if they represent a susceptibility for underlying dysanaptic lung growth in family members of individuals with early-onset COPD, or whether these more sensitive pulmonary function parameters may identify early smoking-related lung damage, to which first-degree relatives of probands with early-onset COPD may be more susceptible. Longitudinal follow-up would be required to determine the underlying pathophysiology. Supported by NIH R01 HL61575, T32 HL07427-22.

Variation in genes of the IL-4/IL-13 pathway: association with early atopic phenotypes in the COAST study. *S. Hoffjan*¹, *D. Nicolae*¹, *J. Gern*², *K. Roberg*², *K. Carlson-Dakes*², *K. Adler*², *C. Tizler*², *S. Sund*², *D. DaSilva*², *R. Hamilton*², *S. White-Gilbertson*², *D. Yoshihara*², *D. Mirel*³, *R. Lemanske Jr*², *C. Ober*¹. 1) Department of Human Genetics, University of Chicago, Chicago, IL; 2) Department of Pediatrics, University of Wisconsin, Madison, WI; 3) Roche Molecular Systems Inc, Alameda, CA.

Asthma is a heterogeneous and multifactorial disorder with symptoms often developing during the first years of life. To assess the contribution of environmental factors in early childhood as well as genetic factors to the subsequent development of asthma, a cohort of children (n=287) at increased risk for asthma and atopic diseases was recruited in the COAST (Childhood Origin of ASThma) study. We studied variation in genes in the IL-4/IL-13 pathway and the expression of various atopic phenotypes in the first year of life in this cohort. 213 COAST children were genotyped for 12 SNPs in the IL4 (-582C/T), IL13 (-1055C/T, 4045C/T, Arg110Gln), and IL4RA (Ile50Val, Asn142Asn, Arg375Ala, Leu289Leu, Cys406Arg, Ser478Pro, Gln551Arg, Ser761Pro) genes. We examined the relationship between individual SNPs and haplotypes with PHA-induced cord blood mononuclear cell IL-13 and IFN- γ responses, the development of atopic dermatitis (AD) and susceptibility to respiratory syncytial virus (RSV) infection in the first year of life. In the IL13 gene we identified a haplotype composed of the -1055C and +4045C alleles and the arginine at position 110, that was associated with reduced IL-13 responses in cord blood (p=0.006), a phenotype characterized by an increased risk of wheezing in the first year of life in this cohort. Further, the IL13 Arg110 allele and the IL4RA Ile50 allele were each associated with increased susceptibility to RSV infection in the first year (p=0.044 and p=0.040, respectively). Lastly, we found an association between the IL4 -590T allele and increased risk of AD (p=0.013). These data suggest that genetic variation in the IL-4/IL-13 pathway may be uniquely associated with the early expression of phenotypic profiles in the first year of life that are known to increase risk for the subsequent development of childhood asthma.

Estimation of genetic risk for bronchial asthma by multiple SNPs in candidate genes. *Y. Suzuki¹, C. Shao¹, F. Kamada¹, M. Tamari², K. Hasegawa², X. Yang¹, Y. Aoki¹, S. Kure¹, K. Kanno¹, X.-Q. Mao³, J.M. Hopkin⁴, T. Shirakawa^{2,3}, Y. Matsubara¹.* 1) Dept of Medical Genetics, Tohoku Univ School of Medicine, Sendai, Japan; 2) Laboratory for Functional Analysis, SNP Research Center, The Institute of Physical and Chemical Research, Yokohama, Japan; 3) Dept of Health Promotion & Human Behavior, Kyoto Univ School of Public Health, Kyoto, Japan; 4) Experimental Medicine Unit, UWS, UK.

Bronchial asthma is a multi-factorial disease caused by interactions between genetic and environmental factors. Several genetic polymorphisms associated with the disease have already been identified, but none of them accounts for a major part of the disease. It seems essential to take multiple polymorphisms into consideration to evaluate the genetic susceptibility for asthma. Here we show a logistic regression approach to integrate multiple single nucleotide polymorphism (SNP) genotypes for understanding and evaluating genetic risk for asthma. We determined 56 SNP genotypes of the candidate genes in 150 adult asthmatics and 150 controls in the English population, and 25 SNPs in 384 adult, 423 child patients, and 335 controls in the Japanese population. To screen SNP-SNP interactions, we performed "two-gene association study" in which an association of one SNP with the disease was assessed after stratification by another SNP's genotype and all the possible two-SNP combinations were tested. Significant SNPs and SNP-SNP interactions were selected with a backward stepwise elimination method in SPSS program. In the English population, the final regression model showed excellent performance. Improvement of $-2LL$ was 129.6 ($P=9.99 \times 10^{-16}$), Nagelkerke's R^2 was 0.502, and c statistic (area under the ROC curve) was 0.867. In Japanese childhood asthma, the performance of the regression was also good, although it was less than that in English. Improvement of $-2LL$ was 83.1 ($P=9.93 \times 10^{-9}$), Nagelkerke's R^2 was 0.167, and c statistic was 0.693. Our results suggested that the logistic regression model is useful in explaining the genetic predisposition to asthma through a set of SNPs of candidate genes. This approach may be applicable for other complex diseases.

Frequency of Human Cytomegalovirus in patients with kidney transplant from Mexican population. R.M.

Hernandez¹, C. Vivas¹, M.P. Gallegos², R. Campos³, A. Celis de la Rosa⁴, S. Ruelas⁵, A.M. Contreras¹ and CMV virus detection. 1) Diagnostico Molecular, IMSS, Guadalajara, Jalisco, Mexico; 2) Medicina Molecular, CIBO, IMSS; 3) Departamento de Medicina Interna; 4) Departamento de Epidemiologia; 5) Unidad de Transplantes, HE, CMNO, IMSS.

Human cytomegalovirus (HCMV) can cause severe morbidity and mortality in allograft and patients kidney transplant development viremia. In our country the kidney insufficiency to produce high rates of mortality by cytomegalovirus and the incidence is unknown. Until a few years ago, HCMV isolation by culture techniques was the only reliable diagnostic method, with as a major drawback the long isolation period (1-8 weeks). Recently, an in vitro DNA amplification technique has been described: the polymerase chain reaction (PCR) with a much higher sensitivity than conventional analysis. We studied the incidence of HCMV in patients with kidney transplant of our population. In 72 patients with kidney transplant was amplified by HCMV PCR. We detected an incidence of 26%, with a tendency to increase after to kidney transplant.

Phylogeny of Fragile X haplotypes from an English population. *S. Ennis*¹, *G. Brightwell*², *A. Collins*¹, *P. Jacobs*², *N.E. Morton*¹. 1) Human Genetics Division, Southampton General Hosp, Tremona Rd., Southampton, Hampshire, England, SO16 6YD; 2) Wessex Regional Genetics Laboratory, Salisbury District Hosp., Salisbury, Wiltshire, England, SP2 8BJ*cis*.

Dynamic expansion of the FRAXA trinucleotide repeat in the FMR1 gene at Xq27.3 causes methylation and transcriptional silencing of the gene. Absence of the gene product FMRP, leads to manifestation of the fragile X syndrome, the most common form of inherited mental retardation in humans. Many factors that predispose to instability of the repeat tract have been identified, but exact determinants of risk remain unclear. Previous linkage disequilibrium studies suggest that *cis*-acting factors may play a role in germline stability.

A panel of 797 independent male chromosomes that span the range of FRAXA repeat sizes have been additionally typed for one microsatellite, three dinucleotide repeats and 11 single nucleotide polymorphisms (SNPs) across 650 kb of the FRAX region. The dinucleotide repeats, DXS548, FRAXAC1 and FRAXAC2, have been used to assign one of 5 haplogroups to each of these chromosomes as previously described (Ennis *et al*, 2001). As would be expected, significant association exists between many of the SNPs and these haplogroups. Although risk of expansion between these haplogroups is variable, within the haplogroups no single SNP appears to be associated with expanded FRAXA alleles. Applying cluster analysis methodology to these 15-marker haplotypes, we have created a dendrogram that represents each of the most closely related haplotypes within this primarily Caucasian population. Evidence suggests that recurrent mutation and/or gene conversion is common in this region, and that variable rates of expansion among clusters within a major haplogroup reflects FRAXA repeat size in founders. The comparatively high risk D haplogroup is the most homogenous group and preliminary evidence also suggests that this group may represent the ancestral haplotype.

Genetic Predisposition to Nuclear Cataract. *A.P. Klein¹, J.A. O'Neill¹, J.E. Bailey-Wilson¹, R. Klein², K.E. Lee², B.E.K. Klein².* 1) Statistical Genetics Section, NIH/NHGRI/IDRB, Baltimore, MD; 2) Department of Ophthalmology and Visual Sciences, University of Wisconsin-Madison Medical School.

Cataract is the most common cause of blindness worldwide. Nuclear cataract is the most common type of age-related cataract and is a late stage of nuclear sclerosis. We assessed the familial aggregation of nuclear sclerosis within data collected as part of the Beaver Dam Eye Study. Previous analysis of these data supported the involvement of a major gene accounting for some of the variability in age-sex adjusted nuclear sclerosis. However these analyses were based upon 1,247 individuals in 564 sibships and therefore did not allow for direct estimation of regressive familial effects including correlations between spouse and between parents and offspring. Follow-up of this cohort identified additional familial relationships, such that data on 2,101 individuals in 620 extended pedigrees with complete age, sex and examination results are now available for analysis. In order to confirm previous results and further examine the influence of additional shared familial effects, other than those due to a major gene, we examined the familial correlations and performed segregation analyses. We confirmed the results of the previous analysis, which found that the inheritance of a major gene accounted for 35% of the variation in adjusted nuclear sclerosis. However, in addition to the effect of the major gene there was also an additional correlation of .16 between parents and offspring. No significant correlation was found between spouses either before or after inclusion of a major gene in the model. The relative ability of both analyses (sibships only and extended pedigrees) to estimate the model parameters will be discussed. The results of the segregation analysis will provide a model for future linkage studies aimed at identifying the genes involved in nuclear cataract development.

Program Nr: 1183 from 2002 ASHG Annual Meeting

Ethnic prevalence of MTRR A66G polymorphism in NW Louisiana pregnant population. *R. Dhanireddy, K. Yanamandra, J.A. Bocchini Jr, T.F. Thurmon, D. Napper, S. Ursin, H. Chen.* Pediatrics, LSU Med Sch, Shreveport, LA.

Since the cloning of human methionine synthase reductase (MTRR) few years ago by Leclerc et al., (1998), limited publications have appeared in the literature on the polymorphism of MTRR gene. Wilson et al. (1999) have first identified the variant A66G in MTRR gene and correlated the variant with neural tube defects (NTD) especially in the pregnant women with low levels of vitamin B12 (cobalamine). The incidence of MTRR 66G allele in the Caucasian population was reported to be 0.51. However, there were no reports on the large scale studies on the incidence of this gene polymorphism in the African-American (AA) population.

Here, we report the incidence of MTRR A66G polymorphism in this ethnic group from 1000 pregnant women. The 66GG mutant homozygosity and allele frequency were 11% and 0.36 respectively, far lower than the Caucasian population (p value <0.0001, odds ratio 0.19, 95% CI 0.12-0.31). Although we have not correlated this polymorphism with NTD in our AA community, this lower mutant gene frequency might reflect the general low incidence of NTD seen in this population. Data, statistics, and ethnic stratification of genotypes from Louisiana will be presented.

NF1-gene analysis based on Denaturing High-Performance Liquid Chromatography (DHPLC). A. De Luca¹, A. Buccino¹, D. Gianni¹, A.I. Lazzarino^{1,2}, M. Mangino¹, S. Giustini³, A. Richetta³, L. Divona³, S. Calvieri³, R. Mingarelli¹, B. Dallapiccola^{1,2}. 1) Istituto CSS - Mendel, Rome, Italy; 2) Department of Experimental Medicine and Pathology, University of Rome La Sapienza, Rome, Italy; 3) Institute of Clinical Dermatology, University of Rome La Sapienza, Rome, Italy.

Neurofibromatosis type 1 (NF1; MIM# 162200) is a common autosomal dominant disorder, characterized by café-au-lait spots, peripheral neurofibromas, Lisch nodules and freckling. The high mutation rate at the NF1 locus results in a wide range of molecular abnormalities. The majority of these mutations are private and rare, generating elevated allelic diversity with a restricted number of recurrent mutations. In this study, we have assessed the efficacy of Denaturing high-performance liquid chromatography (DHPLC), a fast and highly sensitive technique based on the detection of heteroduplexes in PCR products by ion pair reverse-phase HPLC under partially denaturing conditions, for detecting mutation in the NF1 gene. We established theoretical conditions for DHPLC analysis of all coding exons and splice junctions of the NF1 gene using the WAVEmaker software version 4.1.40 (Transgenomic, Crewe, UK) and screened for mutations a panel of 40 unrelated NF1 patients (25 sporadic and 15 familial), genetically uncharacterized. Disruptive mutations were identified in twenty-nine individuals with an overall mutation detection rate of 72,5%. The mutations included eight deletions (exons 4b, 7, 10a, 14, 26 and 31), one insertion (exon 8), nine nonsense mutations (exons 10a, 13, 23.1, 27a, 29, 31 and 36), six missense mutations (exons 15, 16, 17, 24 and 31), four splice errors (exons 11, 14, 36 and 40) and a complex rearrangement within exon 16. Nineteen (65,5%) of the identified mutations are novel. Eight unclassified and three previously reported polymorphisms were also detected. None of the missense mutations identified in this study were found in a screen of 150 controls. Our results suggest that DHPLC provides an accurate method for the rapid identification of NF1 mutations. This work was supported by the Italian Ministry of Health and Education.

Calculating the frequency of (GJB6-D13S1830) and identifying other non-coding GJB2 deafness-causing mutations. *P.G. Chamberlin¹, H. Azaiez¹, S. Prasad¹, R.A. Cucchi¹, F. Moreno², I. del Castillo², R.J.H. Smith¹.* 1) Molecular Otolaryngology Research Laboratories, Department of Otolaryngology, University of Iowa, Iowa City, IA, USA; 2) Unidad de Genetica Molecular, Hospital Ramon y Cajal, Madrid, Spain.

Statement of Purpose. Most mutations at the DFNB1 locus cause autosomal recessive sensorineural deafness (ARSND) by changing the coding sequence of GJB2, the gene that encodes the gap junction protein Connexin-26. Recently, a large deletion 35kb upstream of GJB2 [(GJB6-D13S1830)] was identified and determined to cause ARSND in homozygotes for this mutation and in compound heterozygotes carrying deafness-causing allele variants of GJB2 on the opposite allele. The carrier frequency of the (GJB6-D13S1830) mutation and its overall contribution to the total GJB2 deafness load in the United States is unknown. **Methods Used.** To determine the frequency of the (GJB6-D13S1830) deletion, we screened 49 deaf persons from our hereditary hearing impairment registry who carry a single GJB2 deafness-causing allele variant. We estimated the (GJB6-D13S1830) carrier rate based on the 35delG carrier rate and the relative frequency of these two mutations. We used the 35delG carrier rate to predict missed mutations by calculating the expected number of 35delG carriers in a panel of 602 non-DFNB1 deaf patients. **Summary of Results.** We identified the (GJB6-D13S1830) deletion in 5 persons, making it the sixth most common deafness-causing mutation at the DFNB1 locus (prevalence of 3.42% in persons with GJB2-related deafness). Our calculated carrier frequency of 0.06% in the US population is consistent with our negative screen of 96 controls. In our panel of 602 non-DFNB1 deaf patients, a difference between the observed number of 35delG carriers (21) and the expected number (15) suggests that there are other non-coding deafness-causing mutations of GJB2. **Conclusion.** Many laboratories offer mutation screening of GJB2 as "state-of-the-art" medicine. Our data illustrate the necessity of including a screen for the (GJB6-D13S1830) deletion. It is likely that other mutations exist outside the coding sequence of GJB2. (Supported by R01-DC02842 to RJHS).

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The Contribution of Otoferlin to Autosomal Recessive Non-Syndromic Deafness. *M.R. Avenarius, N.C. Meyer, R.J.H. Smith.* Molecular Otolaryngology Research Laboratories, University of Iowa, Iowa City, IA.

Statement of Purpose. Approximately 50% of the autosomal recessive non-syndromic deafness (ARNSD) is accounted for by mutations at the DFNB1 locus. We hypothesize that mutations at the DFNB9 locus play a moderate role in making up a fraction of the remaining 50% of ARNSD. **Methods Used.** We studied multiplex families segregating ARNSD in whom mutation screening of GJB2 and SLC26A4 was negative. To identify the subset of these families for OTOF mutation screening, we determined haplotype segregation at the DFNB9 locus using tightly linked microsatellite repeat polymorphic markers. Mutation screening of OTOF in selected families was completed by SSCP and bi-directional sequencing. **Summary of Results.** Eighteen multiplex families segregated ARNSD and did not demonstrate mutations in GJB2 or SLC26A4. Of five of these families haplotype segregation in affected persons was consistent with deafness at the DFNB9 locus. In three of these families, mutation screening of OTOF identified allele variants presumed to cause ARNSD. **Conclusion.** We estimate the contribution of OTOF to autosomal recessive non-syndromic genetic deafness at 8%. This contribution is substantial enough to warrant mutation screening of OTOF in children with ARNSD.

GJB2 (Connexin 26) mutations in non-syndromic hearing loss patients from North India. *B. Mittal, M. Mukherjee, R. Puri, S.R. Phadke.* Dept of Genetics, Sanjay Gandhi PostGrad Inst, Lucknow, UP, India.

Non-syndromic hearing loss (NSHL) is the most common form of deafness with a frequency of approximately 1 in 1000 live births. Mutations in the gap junction protein connexin 26 (Cx26) have been established as the basis of autosomal recessive non-syndromic hearing loss (ARNSHL). The Cx26 gene is located at the DFNB1 locus on chromosome 13q12. In caucasian populations a single mutation, 35delG, accounts for most cases of NSHL. We have looked for Cx26 mutations in 55 ARNSHL patients of North India from the local institutions and our genetics out-patient-department. The patients were selected on the basis of their having bilateral, sensorineural, prelingual and non-syndromic hearing loss. Six mutations in the Cx26 gene viz. Gdel 35 bp, G-to-A 71 bp, G-to-A 231 > bp, C-to-T 370 bp, T-to-C 101 bp, 235del C were included in our initial screening programme. Allele-specific primer pairs, normal and mutant were used for PCR amplification of genomic DNA. Out of 55 patients analysed only one patient showed a homozygous mutation of C-to-T, 370 bp. The father of this patient was found to be a carrier for the mutation. Two patients in another family were heterozygous for the mutation G-to-A 71 bp. Based on our present study, we conclude that the common mutation, 35del G is not prevalent in our North Indian population as described in few other ethnic groups in some parts of the world.

A Multiethnic Study of 35delG and 167delT Mutations in the Connexin 26 Gene in Diverse Los Angeles

Population Groups. *P.S. Kim*¹, *G. Manligas*¹, *M. Telatar*², *K.V. Lu*², *R.K. Iyer*², *W.W. Grody*^{1,2}, *L. Schimmenti*¹. 1) Department of Human Genetics, UCLA, Los Angeles, CA 90095; 2) Department of Pathology and Laboratory Medicine, UCLA, School of Medicine, Los Angeles, CA 90095.

Nonsyndromic deafness (DFN) is characterized by mild-to-profound hearing loss with no other associated medical conditions. Over thirty non-syndromic deafness-causing genes have been identified. Among these genes, mutations in *Connexin 26* (*Cx26*) on chromosome 13q11-12 account for approximately 50% of patients with an autosomal recessive form of deafness. Two common mutations, 35delG and 167delT, have been identified in populations with European, Middle Eastern and Ashkenazi ancestry; however, few studies have surveyed an ethnically diverse North American population. Here we report the results of a survey screening for the presence of two common mutant alleles in the *Cx26* gene, 35delG and 167delT, in an anonymized multiethnic cohort of 350 individuals from the Los Angeles area. While we did not detect carriers for these two mutant alleles from Native American (50), Asian American (100), or African American (50) populations; Hispanic Americans (150) exhibited an overall carrier frequency of 2.0%. The mutant 35delG allele was found in 2 of 150 Hispanic American genomic DNA samples analyzed (1.5%), whereas the mutant 167delT allele was detected in one Hispanic American sample (0.5%). Because there are various other highly prevalent mutant alleles commonly found within specific ethnic populations (such as 235delC in Asian populations), and because *Cx26* is a relatively small gene, complete sequencing of the gene could be considered for mutation screening in ethnically diverse populations as technologies become amenable.

Mutation Screening of SLC26A4 by DHPLC. *S.D. Prasad¹, K.A. Kolln¹, R.A. Cucci¹, P. Van Hauwe², P. Coucke², R. Trembath³, G. Van Camp², R.J.H. Smith¹.* 1) Department of Otolaryngology; Interdepartmental PhD Program in Genetics, University of Iowa, Iowa City, IA, USA; 2) Department of Medical Genetics, University of Antwerp, Antwerp, Belgium; 3) Division of Medical Genetics, University of Leicester, Leicester, England.

Pendred's syndrome (PS), an autosomal recessive disease characterized by goiter and congenital sensorineural hearing loss, accounts for approximately 10% of hereditary deafness. Most patients with PS are euthyroid, although they usually have an abnormal perchlorate test reflecting perchlorate-induced inhibition of the sodium/iodide symporter in the thyrocyte and cytoplasmic leakage of iodide back into the bloodstream. The sensorineural deafness is typically associated with inner ear malformations that range in severity from dilation of the vestibular aqueduct to Mondini dysplasia. The PDS gene (SLC26A4) encodes pendrin. Initial predictions of SLC26A4 structure suggested a protein with 11 transmembrane domains and an extracellular carboxy terminus (Everett et al 1997). However, Royaux et al have shown that the carboxy terminus is intracellular implying that an additional alpha helix spans the cell membrane (Royaux et al 2000). Data from 10 transmembrane prediction programs generated results ranging from in 8-13 transmembrane domains, with four programs predicting 12 transmembrane domains. We considered MEMSAT2 results most reasonable based on a recent review ranking this program as one of the most accurate (Jones et al 1994, Simon et al 2001). It predicted 12 transmembrane domains. A wide spectrum of mutations in SLC26A4 has been associated with PS. In this study, we describe our strategy for mutation screening of SLC26A4 using denaturing HPLC. We report the detection of ~42 distinct mutations using DHPLC. In comparison to SSCP, DHPLC is more sensitive. Samples with known mutations were PCR amplified, heteroduplexed, and analyzed under denaturing conditions using the Wave DHPLC system. To achieve a high detection rate, we performed the analysis at multiple temperatures and buffer gradients. Acknowledgements. This research was supported by the Doris Duke Clinical Research Fellowship Program (KAK) and R01-DC02842 (RJHS).

Cytokine gene polymorphism correlates with ischemic cardiomyopathy and rates of rejection episodes in cardiac transplantation. *D.O. McDaniel¹, P. Perrin¹, V. Coleman³, S. Yamout¹, J. Cameron³, C. Moore².* 1) Dept Surgery, Univ MS Medical Ctr, Jackson, MS; 2) Dept Medicine, Univ MS Medical Ctr, Jackson, MS; 3) Dept Biology, JSU, Jackson, MS.

Effectiveness of cytokine gene polymorphisms were evaluated in African-American (AA) and Caucasian (CAU) patients that had undergone cardiac transplantation (CTx). It has been suggested that allograft rejection is mediated by cytokines. Our working hypothesis is that the clinical condition of recipients before CTx might affect the outcome of allograft function through pre-existing genetic factors that are unique to each individual. Genomic DNA samples from 19 AA and 46 CAU recipients were tested by either single or multiplex PCR. Frequency distributions of genotypes were analyzed in respect to pre-CTx clinical characterization including coronary artery disease (CAD) and non-ischemic heart failure. The rejections were scored based on the number of biopsies graded greater than 2. Overall, the IL-10 high producer genotype was present in all patients graded as 1A and was significantly reduced in grade 2, 3A and 3B patients (28.6%, $p=0.02$; 23%, $p=0.001$; and 20%, $p=0.04$, respectively). IFN-g A/A and IL-10 low producer genotypes were significantly increased in AA grade 3A recipients compared to CAU grade 3A recipients (47% vs 16.1%, $p=0.04$; 76.5% vs 32.2%, $p=0.006$ respectively). Both racial groups showed a high frequency of TGF- β 1 high producer genotype compared with controls (AA: 70.5% vs 33.7%, $p=0.006$; CAU: 71% vs 26.2%, $p=0.0001$). There was a direct relationship between IFN-g T/T high producer and ischemic CAD as compared with IFN-g A/A low producer and non-ischemic heart failure, supporting the influence of immunological factors in vasculopathy. In contrast, IL-2 T/T and IL-6 G/G high producer genotypes were present at a high frequency in AA recipients compared with CAU, with notable racial differences. These data indicate the importance that genotype analysis may have in understanding the pathophysiology of CTx outcome and may lead to better treatment options.

Design approach for a database system integrating and interpreting demographic, clinical and biological data. S. Pelletier^{1,4}, J. Lyman^{1,4}, J. Dalton^{2,4}, S. Tropello^{2,4}, B. Dembling^{1,4}, K. Scully^{1,4}, J. Boyd^{3,4}, P. Beling^{2,4}, S. Guerlain^{2,4}, E. Bass^{2,4}, W. Knaus^{1,4}, D. Brown^{2,4}. 1) Dept of Health Evaluation Sci; 2) Systems Engineering; 3) Dept of Pathology; 4) University of Virginia Health System.

The rapid expansion of genetic, disease, health outcomes and treatment information will revolutionize clinical medicine, research and public health if the information can be integrated. Our approach to integration engages system users in the design phase and considers the user interface and required analyses as well as the data to be integrated. As part of a larger project to link demographic, clinical and biological data and provide an interface and tools for complex data analysis, we have assembled an interdisciplinary team (e.g. systems engineering, informatics, clinical, biological, mathematics, public health) for the design phase. This phase encompasses the first steps in the overall integration process; requirements analysis, prototype design and evaluation. Step 1) Requirements analysis: Potential users were asked how the system described could advance their research; data and analytical needs were addressed specifically. We documented their requirements as well as existing data sources and analytical tools. Step 2) Prototype design: Users' requirements drove prototype designs; multiple design alternatives were developed for the system architecture, analytical toolkit, and user interface. Step 3) Evaluation: Prototype designs were evaluated with users and performance matrices. Prototype redesigns continue iteratively with each evaluation. We present the results of the requirements analysis, prototype designs and the initial evaluations. We include key system architecture design findings (e.g. data standards, security, barriers to fulfilling user needs). We describe critical analytical needs identified (e.g. gene expression data analysis) and tool designs to address compliance of user queries to new regulations affecting electronic medical records (HIPAA). Lastly, we describe our approach to the design of a common user interface that addresses the query and data visualization needs of multi-disciplinary users.

Internet-based hereditary cancer susceptibility data system for genetic counseling and research. www.fcgn.org.

R. Sutphen^{1,2,4}, *J. Malloy*⁴, *L. Anderson-Dame*⁴, *J.P. Krischer*^{2,3,4} and Florida Cancer Genetics Network (FCGN). 1) Dept Clinical Genetics, #7815, All Children's Hosp, St Petersburg, FL; 2) Departments of Interdisciplinary Oncology and Pediatrics, University of South Florida College of Medicine, Tampa, FL; 3) Department of Computer Science and Engineering, University of South Florida College of Engineering, Tampa, FL; 4) H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL.

We describe an internet-based system for cancer genetics risk assessment counseling, family pedigree illustration and research registry participation. The Florida Cancer Genetics Network (FCGN) automates collection of the family and personal medical history information required for these processes. Data may be 1) entered online or 2) entered on paper forms that can be faxed into a web server for direct (automated) data entry accomplished within minutes. Data is then available for viewing, editing and printing via a secure website. The system generates a family pedigree and risk calculation that can also be viewed or printed from the website. For research initiatives, data in the system can easily be queried to determine the number of individuals available who meet specific eligibility requirements. Authentication and authorization features allow easy access to all data for which the user has permission, while restricting all other data from access. Web access to the system requires a standard web browser (such as Microsoft Internet Explorer version 5.5 or higher) and use of free encryption software available on the internet (128-bit Secure Sockets Layer encryption). The system has two main uses - 1) it accomplishes the data collection, pedigree-drawing and risk assessment procedures of clinical genetic counseling for hereditary cancer susceptibility quickly and easily and 2) it automates enrollment of individuals with high cancer risk in a registry designed for individuals who are interested in participating in cancer research studies. The internet-based design of this system makes it accessible by cancer genetics centers around the world. To our knowledge, there is no comparable system in current use.

Prototype microfluidic devices for cell fractionation and isolation. *M. Caggana¹, A.P. Russo¹, A.J.H. Spence², S.T. Retterer², S.J. Duva¹, D.H. Szarowski¹, M.S. Isaacson², J.N. Turner¹, L.D. McCurdy¹.* 1) Wadsworth Ctr, New York State Dept Health, Albany, NY; 2) Departments of Applied Physics and Biomedical Engineering, Cornell University, Ithaca, NY.

The miniaturization of laboratory techniques and equipment for biological assays has become a dominating focus in research. The development of micro-total analysis systems (mTAS) has made striking progress but still remains a serious challenge. Several prototype devices have been described that perform analytical reactions and detection on a single platform. With few exceptions most devices described do not integrate sample preparation as a part of on-chip analyses. Sample preparation is often the most difficult step in an assay, especially when working with complex heterogeneous mixtures such as whole blood. The separation of cells from a complex suspension is desired for many purposes. Most of these applications can be divided into two categories: (1) depletion of undesired cell types from a population of desired cells (bone marrow purging for the removal of residual cancer cells) or (2) positive selection of a cell type for further analysis (isolation of fetal cells from maternal circulation). Using photolithography, replica molding, and soft lithography, we have constructed microfluidic devices to separate heterogeneous mixtures of cells. Device design is based upon arrays of microfluidic channels with decreasing widths; as cells navigate through the device they deform to either negotiate the microfluidic channels or are impeded by the rigid structure. Using human whole blood spiked with neuroblastoma cells, we demonstrate that normal red and white blood cells traverse the device at different rates while the cancer cells are retained; future device generations will incorporate components for on-chip genetic analyses. Selective capture and enrichment of neuroblastoma cells from peripheral circulation may allow for improved cancer detection and permit the discrimination between clinically favorable or unfavorable disease states. In addition to genetic diagnostic and/or prognostic purposes, this prototype device may prove useful for bone marrow purging prior to autologous transplantation.

PCR dosage-based testing for genes spanning the Williams-Beuren syndrome critical region. *M.J. Somerville, M. Hicks, R. Tomaszewski, K.A. Sprysak, B.G. Elyas, A.Y. Ng, S.M. Haase, L.M. Vicen-Wyhony.* Dept Medical Genetics, Univ Alberta, Edmonton, AB, Canada.

Deletions at the 7q11.23 locus have been identified in the vast majority of patients with Williams-Beuren Syndrome (WBS). This contiguous gene deletion disorder is defined by a number of clinical features including infantile hypercalcemia, supraaortic stenosis, and elfin facies. Routine testing for this condition involves cytogenetic fluorescence in situ hybridization (FISH) using a probe from the 7q11.23 deleted region. This method is however, relatively time-consuming and expensive, and may be of limited sensitivity. We have undertaken an assessment of deletion status in a total of 62 index cases, referred for confirmation of a tentative diagnosis of WBS from a wide variety of clinical sources. A real-time PCR assay was developed using multiplex quantitative analysis for sequences within unique open reading frame segments of the *ELN*, *LIMK1*, and *GTF2I* genes. Hemizygous deletions of *ELN* and *LIMK1* have been implicated in typical WBS. Isolated deletion of *GTF2I* resulting from inversion of the WBS critical region has been hypothesized as a possible cause of some atypical WBS cases (Osborne, et al. 2001). We observed a total 6 of 62 (9.7%) cases with hemizygous deletions that encompassed all 3 genes, and these findings were confirmed by cytogenetic FISH analysis. No isolated deletions of *GTF2I* were detected in our 62 index cases. The lack of isolated *GTF2I* deletions may be due to our limited sample size, or undefined diagnostic criteria for referral. We are currently screening a larger population of typical and atypical WBS cases using PCR dosage-detection of these 3 genes, in an effort to determine the frequency of isolated gene deletions within the WBS critical region. According to our findings, integration of molecular and cytogenetic services using direct DNA-based analysis results in higher throughput, substantial reduction of cost and turn-around-time, and potentially increased sensitivity for detection of WBS deletions. Osborne, et al. (2001) Nat. Genet. 29: 321-325.

MTHFR 677CC but not MTRR 66AA genotype may protect against Multiple Myeloma and Solid tumors. K.

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Multiple myeloma (MM) is characterized by neoplastic proliferation of a single clone of plasma cells which produces a monoclonal immunoglobulin. It accounts for one percent of all malignant diseases and more than ten percent of hematologic malignancies. Though MM is of unknown etiology, structural changes in chromosomes 1, 11, and 14 (frequently in 14q32), monosomies and trisomies, and translocations have been observed. Alterations in the expression of c-myc and H-ras have been reported.

Folate deficiency has been suspected of tumorigenesis of solid tumors such as breast, cervix, colon, pancreas, and liver, but there has been little study of enzyme gene polymorphisms in the folate and cobalamin (vitamin B12) pathway such as methylenetetrahydrofolate reductase (MTHFR) C677T, methionine synthase reductase (MTRR) A66G, and methionine synthase A2756G.

We have studied MTHFR and MTRR polymorphisms in cancer patients. Initial results of studies of one hundred patients suggest significant negative correlations of MTHFR 677CC normal genotypes with MM (Chi square 9, p value <0.003, odds ratio of <0.3 with a 95%CI of 0.1-0.7), and with solid tumors (Chi square 4, p value <0.05, odds ratio 0.4 with a 95%CI of 0.3-1), indicating protection by normal genotype. There was no correlation with the MTRR 66AA normal genotype. This being the first report of protection of 677CC normal homozygotes against MM and solid tumors, experience from other investigators would be helpful. Data on statistics, ethnic stratification of MTHFR and MTRR genotypes on various cancers and controls will be presented.

Hereditary Colorectal Cancer Registries: Current Status and Practices. *W.K. Kohlmann¹, C.H. Solomon², B. Allen³*. 1) Division of Molecular Medicine & Genetics, University of Michigan, Ann Arbor, MI; 2) University of Utah Huntsman Cancer Institute, Salt Lake City, UT; 3) Comprehensive Cancer Center & Department of Medicine, University of California-San Francisco, San Francisco, CA.

The Collaborative Group of the Americas on Hereditary Colorectal Cancer (CGA) was established to facilitate research, educate health care professionals, and provide guidance and support for hereditary colorectal cancer registries. As part of the 2001 annual CGA conference a survey assessing organizational structure, patient populations, consenting practices, family expansion strategies and IRB involvement was performed to determine the current status and practices of hereditary colorectal cancer registries. Twenty-five registries were identified through the CGA and a study announcement was posted on the National Society of Genetic Counselors' listserv. Twenty-three responses were received, 20 from CGA registries and 3 from registries identified through the listserv. Twenty-one were established registries, with an average of 9.4(range 1-31 years) years in operation, and 2 were in existence for less than 1 year. The 23 registries had different functions, with 18 (78.3%) providing clinical services and conducting research and 4 (17.4%) for research only. Seventeen registries responded to the following questions about consenting, confidentiality, and family expansion. Fourteen (82%) required written consent for registry enrollment, and 15 (88.2%) registries were IRB approved protocols. All 17 responding registries reported taking measures to protect patient confidentiality. Common measures included staff certification by the NIH Protection of Human Research Subjects Research module, certificate of confidentiality, use of numerical identifiers, and restriction of access to genetic testing information. Fourteen of the 17 responding registries (82.3%) obtained consent from the proband to contact extended relatives. Three registries reported that rather than actively contacting relatives based only on the probands consent, family members were required to contact the registry directly if they wanted to participate. Data from this study will serve as a guide for the development of new registries.

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Reverse-hybridization analysis of multiple genetic risk factors for cardiovascular disease. *C. Oberkanins, A. Moritz, F. Kury.* ViennaLab, A-1110 Vienna, Austria.

Background: A number of genetic and environmental risk factors have been found or suspected to predispose to cardiovascular disease (CVD), the term collectively used for disorders of the heart and blood vessels. Inherited susceptibility may be caused by mutations and polymorphisms in a variety of genes involved in blood coagulation, regulation of blood pressure, and metabolism of lipids, glucose, homocysteine or iron. **Objectives:** Our aim was to design and evaluate an assay capable of simultaneously analyzing multiple candidate markers for genetic CVD risk by reverse-hybridization. **Methods:** Two multiplex PCR reactions were set up to amplify and biotinylate DNA fragments covering the following 12 mutations: FV R506Q (Leiden), FV H1299R (R2), Prothrombin G20210A, MTHFR C677T, MTHFR A1298C, b-Fibrinogen -455 G-A, PAI-1 4G/5G, Factor XIII V34L, GPIIIa L33P (HPA-1), HFE C282Y, Apo B R3500Q, Apo E2/E3/E4. After careful selection of oligonucleotides specifically recognizing wild-type or mutant sequences, membrane teststrips were fabricated presenting a parallel array of allele-specific probes for each of the CVD risk factors. For a sample to be analyzed DNA has to be isolated and in vitro amplified, and PCR fragments are hybridized under exactly defined stringency conditions to a teststrip and detected by enzymatic color reaction. **Results and Conclusions:** We have developed a reverse-hybridization assay (CVD StripAssay) for the rapid and simultaneous genotyping of candidate CVD risk factors. The protocol was set up and validated using pretyped human DNA samples, as well as recombinant plasmid clones containing the mutant alleles as confirmed by DNA sequencing. The entire genotyping procedure from blood sampling to final result requires less than 6 hours, and hybridization and detection may be fully automated on existing equipment (e.g. TECAN profiBlot). The test is simple and convenient, requires very small amounts of samples, and can easily be modified to include additional markers of interest. (oberkanins@viennalab.co.at).

Is hyperhomocysteinemia a risk factor in preeclampsia for Korean pregnant women? *Y.J Kim^{1,3}, H.S Park^{2,3}, E.H Ha^{2,3}, W.K Kim⁴, N.S Chang⁵*. 1) Dept of OB/GYN, MokDong Hosp, Ewha Womans Univ, Seoul, Korea; 2) Dept of Preventive Medicine, Ewha Womans Univ, Seoul, Korea; 3) Medical Research Center, Ewha Womans Univ, Seoul, Korea; 4) Dept of Food Science and Nutrition, Dankook Univ, Seoul, Korea; 5) Dept of Nutritional Science, Ewha Womans Univ, Seoul, Korea.

Objective: The purpose of this study was to determine if an elevated plasma homocysteine level at the time of delivery is associated with the development of preeclampsia and to investigate whether 677(C->T) polymorphism in the 5, 10-methylenetetrahydrofolate reductase(MTHFR) gene, low folate status, and low vitamin B12 levels are risk factors for the development of preeclampsia in Korean pregnant women. Study Design: DNA was extracted from whole blood of 191 healthy pregnant women and 84 preeclampsia patients. All samples were genotyped for the 677(C->T) polymorphism in MTHFR gene by polymerase chain reaction followed by restriction fragment length polymorphism analysis(PCR-RELP). Serum levels of homocysteine, folate, and vitamin B12 were measured; by high performance liquid chromatography for homocysteine, and radioassay for folate and vitamin B12. Results were analyzed with the Chi-square test, ANOVA, and the logistic regression analysis. Results: Women with severe preeclampsia showed higher concentrations of serum homocysteine(10.5mmol/L) than healthy pregnant women(8.46mmol/L) and women with mild preeclampsia(8.24umol/L)(p=0.09). For serum folate levels, women with severe(12.7ng/ml) or mild(13.2ng/ml) preeclampsia showed increased levels than healthy pregnant women (9.23ng/ml) (p=0.0046). Hyperhomocysteinemia of homocysteine levels >14mmol/L was associated with preeclampsia(odds ratio=2.36, 95%CI: 1.09, 5.12). Conclusion: These findings suggest that hyperhomocysteine in pregnancy may be a risk factor for preeclampsia.

Folate and Cobalamin metabolizing enzyme gene polymorphisms and Orofacial Clefts in West Africa. *T.F. Thurmon¹, K.M. Layton², K. Yanamandra¹, D. Napper¹, S. Ursin¹, H. Chen¹, R. Dhanireddy¹, J.A. Bocchini Jr¹.* 1) Dept Pediatrics-Genetics, Louisiana State Univ, Shreveport, LA; 2) Mercy ships, Lindale, Texas.

Orofacial clefts (OFC) are multifactorial genetic defects consisting of cleft lip (CL) with or without cleft palate CL(P), and cleft palate (CP). There are syndromic and non-syndromic forms. One of the environmental factors responsible for these conditions is nutrition. Previous studies have revealed periconceptional supplementation of multivitamins, folic acid in particular, reduce the recurrence risk of neural tube defects (NTD). There have been reports that high doses of folate reduce the recurrence risk of OFC also. Gene polymorphism in 5,10-methylenetetrahydrofolate reductase (MTHFR) the rate limiting enzyme in the metabolism of folate in the C1 carbon-pathway has been implicated in the etiology of OFC. MTHFR 677TT homozygosity results in the generation of homocystinemia, and has been reported to be increased in patients with OFC. Methionine synthase reductase (MTRR) regenerates inactive methionine synthase-cobalamin II by reductive methylation to active methionine synthase-methylcobalamin III which participates in the regeneration of homocysteine to methionine. Thus vitamin B12 metabolism is part of folate pathway and provides methyl groups in the methylation of DNA. MTRR A66G polymorphism is reported to be involved in NTD and chromosomal defects. However, its involvement in OFC is unknown.

We are studying MTHFR C677T and MTRR A66G genotypes in patients with birth defects including OFC, NTD and chromosomal defects. We have genotyped fifty three West African patients with OFC and seventy seven controls. Data from our pilot studies suggest that neither MTHFR C677T (Chi square 1.1, p value 0.3, odds ratio 0.4 with 95% CI 0.1-1.7) nor MTRR A66G (Chi square 0, p value 1, odds ratio 1.3 with 95% CI of 0.2-11.7) polymorphisms are risk factors in the development of OFC. Our data about the MTRR polymorphism in OFC is the first to be reported. Data on statistics, MTHFR and MTRR genotypes in various OFC will be presented.

Framework for assessing impact and identifying public health interventions for severe combined immunodeficiency (SCID). *L.V. Kalman¹, M.L. Lindegren¹, L.J. Kobrynski², C.A. Moore¹, S.A. Rasmussen¹, R. Vogt¹, T. Spira¹, S. Grosse¹, M. Gwinn¹, R. Buckley³, M.J. Khoury¹.* 1) NCEH, Centers for Disease Control and Prevention, Atlanta, GA; 2) Emory University, Atlanta, GA; 3) Duke University, Durham, NC.

Background: In November 2001, CDC convened a panel of experts to apply a public health approach to primary immunodeficiency (PI) diseases, a group of 100 immune disorders that includes SCID. We applied a framework for assessing impact and identifying public health interventions to prevent morbidity, disability, and mortality to SCID. The framework has 4 components: epidemiologic assessment; evaluation of diagnostic testing; development, implementation and evaluation of population interventions; and information dissemination. **Assessment:** Current disease/mutation registries and surveys provide incomplete data on incidence, prevalence, and natural history. **Interventions:** SCID is characterized by profound deficiencies of T and B cell function. SCID is fatal in infancy unless identified and treated with a hematopoietic stem cell transplant. Research suggests that transplantation during early infancy can lead to higher survival rates. Public health intervention for SCID may be possible through newborn screening. **Testing:** Potential tests for population (newborn) screening could include quantitative T-cell tests. Data from one center show that low total lymphocyte counts at birth would identify at least 80% of SCID patients, and that testing for T-cell lymphopenia (TCLP) would identify almost all. TCLP potentially could be detected in dried blood spots collected routinely for newborn screening. A screening test may involve measuring soluble T-cell specific biomarkers (CD3 or CD4) that correlate with absolute T-cell counts. Potential tests must be evaluated for analytic and clinical validity and utility in pilot population-based studies. **Dissemination:** We reviewed strategies to increase awareness of PI. Population-based data are needed to assess the public health burden of SCID and effects of early intervention. Validated tests and pilot population studies are necessary to determine the potential of newborn screening for SCID.

CLINICAL AND MOLECULAR ANALYSIS OF THE FRAGILE X SYNDROME IN MENTALLY RETARDED MALES FROM THE VENEZUELAN NORTHWESTERN REGION. *W.M. Zabala, N. Nava, S. Gonzalez, W. Delgado, L. Borjas, E. Fernández, A. Rojas, L. Pineda.* Unidad de Genética Médica, Universidad del Zulia, Maracaibo, Zulia State, Venezuela.

The fragile X syndrome (SFX), is an atypical X-linked dominant inheritance condition. The prevalence of this disease is 1/4000 males and 1/6000 females. Affected males show a fragile site at Xq 27.3 (FRAXA), is usually caused by a trinucleotide (CGG)_n expansion (>230) in the first exon of the FMR1 gene. Expanded alleles are methylated at a CpG island just proximal to FMR1 gene and are expressed. A more distal fragile site, FRAXE, has been identified. Is associated with amplification of (CGG)_n repeat (6-25 normal range, up to 200, fully mutation). The aim of this study was the clinical and molecular evaluation of 98 males with mental retardation of unknown cause in patients with retarded mentally in Zulia state of Venezuela. A 13-item checklist (Hagerman,1987) was used. A total score of 16 or higher had a significant yield of fra(x) positive patients. PCR analysis was performed according to the method of Wang et al. Our studies showed that the proportion of patients with FRAXA in this sample is 4.08% (4/98). It was not significantly different to that reported in most populations (3-6%). PCR analysis detecting methylation of the CpG island confirmed the presence of an expanded FRAXA allele. The proportion of mental retardation associated with FRAXE was 1.02% (1/98). These results suggest that FRAXE is not a common etiological factor among this group of patients. The SXF is the second cause of RM. The prevalence of SXF in the Venezuelan population is unknown. The non-radioactive PCR appears to be a reliable, cheap and efficient test for initial screening of samples for the presence of FRAXA and FRAXE mutations.

MTRR A66G polymorphism and Prenatal risk for NTD and Chromosomal defects. *H. Chen, K. Yanamandra, D. Napper, S. Ursin, T.F. Thurmon, R. Dhanireddy, J.A. Bocchini Jr.* Dept Pediatrics, Perinatal Gen, LSU Health Sci Ctr, Shreveport, LA.

We have shown previously that the C677T polymorphism in the gene of methylenetetrahydrofolate reductase (MTHFR), a folate metabolizing enzyme, was associated with prenatal risk of birth defects. There was an increase in mutant 677TT homozygosity in the prenatally identified at risk women for neural tube defects (NTD) and chromosomal defects (CD) identified through our prenatal triple (MSAFP, MShCG, and MSuE3) screening program. We have also reported an association of MTHFR C677T polymorphism with abnormal fetal karyotypes other than Down syndrome and no association with Down syndrome.

In the present investigation we are studying the association of methionine synthase reductase (MTRR, a cobalamin metabolizing enzyme in folate pathway) A66G polymorphism with prenatal NTD and CD risks. We genotyped over one thousand pregnant women from our prenatal maternal serum screening program who were screened for NTD and CD using triple biochemical analyte method.

Although there were higher heterozygotes in the high risk CD group and similar number in NTD group, compared to controls, percent mutant allele was same in both cases and controls. Our results do not seem to support the findings from literature and do not indicate that MTRR polymorphism was a risk factor in the prenatal development of either NTD or CD. Data and statistics and ethnic stratification of genotypes among pregnant women with high and low prenatal risk for birth defects will be presented.

The practical aspects of integrated prenatal screening. CA. Gibbons¹, SA. Farrell², J. Kennedy¹, T. Huang¹, C. Meier¹, PR. Wyatt¹, AM. Summers¹. 1) Genetics Program, North York General Hospital, Toronto, Ontario, Canada; 2) Genetics Program, The Credit Valley Hospital, Mississauga, Ontario, Canada.

We review successes and problems of Integrated prenatal screening (IPS) in a community setting. IPS combines first trimester screening by ultrasonography for nuchal translucency (NT) and pregnancy associated plasma protein-A (PAPP-A), with second trimester biochemical screening by alpha-fetoprotein (AFP), unconjugate estriol (uE3), and human chorionic gonadotrophin (hCG). It gives the estimate of the risk of Down syndrome (DS) at the completion of all components. IPS is expected to detect 85% of cases of DS at term with a false positive rate of 1.5% at a risk cut-off of 1/200. Prior to implementation, there were concerns about a potential high drop out rate because of withholding results of first trimester screening until all assays are completed. In practice, drop out does not appear to be a major issue however limited availability of NT, discrepancies in pregnancy dating and sample misdirection can create problems for health care practitioners. In our IPS program, 6942 women have started IPS. 6579 women completed IPS, giving a drop out rate of 5.2%. 96 of the 363 dropouts had amniocentesis. The outcome of the 267 pregnancies is unknown. It would be expected that some miscarried. One problem has been accurate gestational dating. Discrepancies in dating from ultrasound laboratories resulted in women presenting at the wrong gestation for screening. This problem could be eliminated by using a single gestational dating source. In Ontario, the Daya' 93 crown-rump curve is used since it best fits Ontario data, based on a separate study. The major sample handling error occurs when the second blood sample is sent to a different laboratory. The problem can be reduced by use of the correct requisition by the physician, attention to laboratory information by transportation staff, and cooperation among laboratories. While there are some logistic problems with IPS, it appears that the benefits outweigh the inconvenience and women and physicians are willing to undertake this screen.

Cytogenetics and Prevalence of Down Syndrome: A Study from Saudi Arabia. *I.M. Amir¹, K. Al-Tawil², A. Al-Harbi²*. 1) Dept Pathology, King Fahad Natl Guard Hosp, Riyadh, Saudi Arabia; 2) Dept Pediatrics, King Fahad Natl Guard Hosp, Riyadh, Saudi Arabia.

Studies on Down syndrome in Saudi Arabia and other Arab countries are sporadic. We report the cytogenetic and prevalence findings of Down syndrome from 277 affected patients investigated at King Fahad National Guard Hospital, Riyadh, Saudi Arabia. The classical trisomy 21 non-disjunction type of Down syndrome was found in 264 patients (95.3% of cases), two of them were mosaic (0.72% of cases). Five patients had maternally inherited types of trisomy 21 that resulted from t(13;21), t(14;21) and t(15;21). Seven patients showed trisomy 21 karyotypes as a result of the i(21q) abnormality and one patient had a rare type of trisomy 21 that resulted from psu idic(21)(q22). Parental blood lymphocyte karyotypes of the last eight patients were normal. Two of the patients with i(21q) were monozygotic twins. One patient with i(21q) also has an older sibling with the classical non-disjunction type of Down syndrome, an indication for possible parental gonadal mosaicism. Out of the 277 patients, 145 were born at King Fahad National Guard Hospital between 1992 and 2001 among a total of 61861 live births. The prevalence rate of Down syndrome during this ten-year period was therefore estimated at 1 in 427 in the absence of an antenatal screening program for Down syndrome. Advanced maternal age at birth of Down syndrome patients was generally evident. Although the cytogenetic pattern of Down syndrome patients in our study is generally similar to that of major international studies, the incidence of this disease is distinctly higher. Currently, there is no official registry of Down syndrome patients in Saudi Arabia, however, in a country of about 21 million people with 3.1% annual population growth the number of Down syndrome patients will inevitably be very high and this emphasizes the need for an effective long-term antenatal screening program. Further studies are in progress to describe the clinical findings of Down syndrome patients in Saudi Arabia and to investigate the correlation between the incidence of Down syndrome and paternal age and maternal parity size.

Is Methionine synthase reductase 66G mutant genotype a risk factor for chromosomal defects? *S.A. Ursin¹, K. Yanamandra¹, I. Gadi², H. Chen¹, T.F. Thurmon¹, D. Napper¹, R. Dhanireddy¹, J.A. Bocchini Jr.¹*. 1) Pediatrics, Louisiana State Univ, Shreveport, LA; 2) LabCorp of America, Research Triangle park, North Carolina.

For a number of years, we have been studying the correlations between gene polymorphisms and genetic diseases such as single gene, multifactorial, and chromosomal disorders (CD). In recent studies of the MTHFR C677T polymorphism in pregnancies at risk for neural tube defects (NTD) or CD, we have found MTHFR 677TT homozygosity to be a risk factor for fetal chromosomal defects other than Down syndrome, with two-fold higher homozygosity over controls (24% vs 12%, p value <0.03, odds ratio 4.7). Fetal trisomy 18 amnios displayed four-fold higher homozygosity over controls (57% vs 12%, p value <0.02, odds ratio 12.6).

Because folate and cobalamin (vitamin B12) share similar pathway, we also studied the methionine synthase reductase (MTRR) A66G polymorphism in 50 amniocyte samples with abnormal karyotypes (trisomies - sex and autosomal, triploidies, translocations and deletions; half Down syndrome and half non-Down syndrome). Our data revealed that there was no correlation between the MTRR A66G polymorphism and the CD (Chi square 0.7, p value 0.4, odds ratio 0.8 with 95% CI 0.5-1.3), in contrast to reports in the literature.

Preliminary studies revealed a fifty percent increase in MTRR 66AG heterozygosity in Down syndrome fetuses, the significance of which is unknown. Data, statistics, and ethnic stratification of genotypes will be presented.

Medical Geneticists Duty to Warn At-Risk Relatives for Genetic Disease. *M.J. Falk^{1,2}, R.B. Dugan¹, M.A. O'Riordan², A.L. Matthews¹, N.H. Robin^{1,2}.* 1) Depts of Genetics; 2) and Pediatrics, University Hospitals of Cleveland, CWRU Sch Med, Cleveland, OH.

When a patient refuses to notify relatives who are at-risk for a genetic condition, genetics professionals are met with conflicting ethical obligations: the duty to respect and protect their patients' right to privacy and confidentiality vs. the duty to prevent harm. The latter duty suggests a responsibility to warn at-risk relatives of their genetic risk, even without their patient's consent. Little investigation has occurred into how genetics professionals view disclosure of genetic test results to at-risk relatives without patient consent. Prior studies have been limited to identifying motivating factors underlying genetics providers predicted responses in hypothetical scenarios. Previously, we examined the actual clinical experience of genetic counselors (GC) with this issue (Nation et al., in press). To further investigate this issue, we surveyed medical geneticists (MG) on their experiences warning at-risk relatives. A questionnaire was sent to 800 board-certified MG, with a 27% (213/800) response rate. 70% (148/213) believe they do bear responsibility to warn at-risk relatives, but most incorrectly answered questions on existing laws and guidelines on this subject. 60% (127/210) reported having had a patient refuse to tell their at-risk relatives. 33% (39/118) described 70 cases in which they seriously considered disclosure without patient consent. Only 4 proceeded to warn at-risk relatives in 5 cases. MG identified patient confidentiality and legal liability as major factors leading to non-disclosure. In Nation et al., GC cited emotional issues as key factors in their decisions not to warn. This study of MG and the previous one on GC show both that do encounter this scenario in clinical practice (60% & 46%, respectively). While disclosure to at-risk relatives without consent was rarely the outcome for either group, differences do exist in how MG and GC approach this delicate situation. The creation of guidelines that take into account the differing perspectives identified in this study would benefit future genetics practitioners faced with this dilemma.

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Development of a database to assess efficacy of pedigree analysis in genetics patients referred for routine indications. *W.S. Meschino, M. Care, E. Wen, L. Velsher.* Dept Genetics, North York General Hosp, Toronto, ON, Canada.

Our Genetics Program serves a large multicultural prenatal population, with over 3300 patient visits per year. The majority of referrals are for advanced maternal age (AMA) or a positive maternal serum screen (MSS), where the average risk of chromosome abnormalities is 0.7 - 1%. A detailed pedigree and reproductive/prenatal history are taken on all routine prenatal patients, a practice widely endorsed by the WHO and others. Through this process, the potential exists to uncover genetic or teratogenic risks that are above those associated with the reason for referral. These risks may be as high as 25 or 50%, much higher than the typical risks associated with AMA or a positive MSS. The pedigree component of the average genetic counselling session is substantial (20 minutes), totalling 1100 hours annually. How often are significant genetic disorders or other risk factors uncovered, which influence the choice of diagnostic tests or outcome of prenatal diagnosis? We present details of a database that was developed to answer these questions. The information recorded includes the patient's risk for a chromosome abnormality or open neural tube defect, as well as details regarding the prenatal history: maternal health and exposure to teratogens. Significant details of the pedigree, and results of all prenatal tests, including carrier screening for high-risk ethnic groups for the patient and her partner were also included. The database captures how often additional tests are offered and how often they are accepted. It was used in a retrospective analysis of 1000 prenatal genetics charts to assess the cost-effectiveness of routine pedigree analysis. It can also be adapted for prospective use.

Informed Decision-Making in Newborn Screening: Highly Variable Regulatory Language. *M.H. Lewis¹, L. McCabe^{1,2}, E.R.B. McCabe^{1,2,3}*. 1) Department of Pediatrics and; 2) Department of Human Genetics, David Geffen School of Medicine at UCLA, Los Angeles, CA; 3) Center for Society, the Individual and Genetics, UCLA, Los Angeles, CA.

Personal autonomy is a fundamental ethical concept and informed decision-making is one mechanism to support personal autonomy. The informed consent process is the formal vehicle for informed decision-making and is generally required prior to a medical test or procedure. The objective of this study was to evaluate the regulatory language governing informed decision-making in state newborn screening programs. To evaluate current practices regarding informed decision-making for newborn screening, statutes from all fifty states plus the District of Columbia, were analyzed. We found that these 51 jurisdictions address issues of informed decision-making for newborn screening in a highly variable fashion. Forty-four programs require mandatory newborn screening. Nine programs exempt newborns from screening based solely on parental objections. Twenty-six programs allow exemptions for religious reasons. Violation of newborn screening requirements is a misdemeanor punishable by fines in six jurisdictions, and by imprisonment in one jurisdiction. Three jurisdictions prohibit testing unless the parent is fully informed of the test purpose. Only five programs require informed consent from parents prior to newborn screening. Eight jurisdictions specifically exclude newborn programs from informed consent requirements for genetic testing. We conclude that the disparate manner in which informed consent issues are treated highlights the need for further study of, and model regulatory language for, informed decision-making. The completion of the Human Genome Project will provide tremendous potential to apply new technologies in newborn screening and expand test batteries. Use of stored newborn screening samples to develop these tests also should be addressed. The overarching goal should be to educate the public and professional communities about the purposes of newborn screening programs and the use of information obtained by newborn screening.

Unanticipated outcome from recent guidelines for CF carrier testing. *P.P. Hawley¹, R.B. Parad^{1,2}, A. Comeau², M.B. Irons¹.* 1) Childrens Hosp, Boston, MA; 2) UMass Medical School, Worcester, MA.

Recent ACMG guidelines for CF carrier testing include recommendations for reflexive testing for the poly T variant in intron 8 when the R117H mutation is detected. In keeping with these guidelines, poly T testing has been offered to R117H carriers seen in the Children's Hospital Genetics Program since 8/1/01. Carriers of R117H include newborns identified through the Massachusetts CF Newborn Screen (CFNS), as well as a parent of the newborn.

11 newborns with elevated IRT levels (10 females, 1 male) were identified with 1 copy of the R117H mutation. 5 with normal sweat tests were homozygous for the 7T allele, including the 1 male. One had a 7T allele trans to an R117H-5T allele. The remaining 5(4 normal, 1 indeterminate sweat test) had a 5T allele trans to an R117H-7T allele. For 2 of 5 newborns homozygous for the 7T allele, the possibility existed for a 5T allele trans to the R117H mutation in sibs. As for the newborn's parents, the R117H mutation was found in 7 fathers and 4 mothers, none of whom had a 5T allele trans to the R117H mutation. For 7 of 11 newborns found to be R117H carriers following CFNS, a discussion ensued with parents concerning variable phenotypes associated with a 5T allele trans to a CF mutation. Phenotypes include: asymptomatic; CBAVD(males); chronic pancreatitis; atypical or rarely classical CF. Relative risk for these outcomes is unknown. In view of possible CF related symptoms in persons with this genotype, consultation in a CF Program is recommended, with any ongoing monitoring to be determined by CF specialists.

The R117H mutation produces CFTR protein with some function. It is conceivable that more severe phenotypes may be seen more frequently when a 5T allele is trans to severe CF mutations. Yet these individuals are not being identified or offered consultation in a CF Program because ACMG guidelines do not recommend poly T testing for all identified carriers. Thus, in carriers identified through CFNS, adhering to guidelines regarding poly T testing of R117H carriers has inadvertently resulted in offering different levels of service to patients with different mutations.

Ethical Issues in Pre-symptomatic Genetic Testing: A Survey of Physicians' Knowledge and Attitudes in Taiwan.

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With the advent of genetic services in Taiwan, a variety of genetic tests are now available for prenatal diagnosis, carrier detection, and pre-symptomatic diagnosis of neuro-degenerative disorders. However, many problems related to ethical issues still exist, such as who should be tested, the process of informed consent prior to testing and methods of dealing with the test results. To understand the physicians' attitudes towards ethical issues, we conducted a survey among physicians focusing on pre-symptomatic testing for neuro-degenerative disorders. A self-administered questionnaire with 10 questions and a case scenario related to Huntington's chorea were used to test the surveyees' opinions about informed consent, children/fetal rights, and information disclosure in the process of genetic counseling. Results were obtained from 61 physicians in tertiary medical centers and community hospitals, including 28 pediatricians, 19 obstetricians and 14 neurologists. Ninety percent of the physicians followed the process of informed consent. With respect to autonomy, although 24.6% of the physicians stated that they would respect the child's decision, 60% considered parents to have the right to know their fetus's condition and would agree to perform prenatal testing on the fetus. However, some inconsistency exists in the fact that 52% of the physicians did not agree on termination of pregnancy if the fetus were found to be affected. While 64% of physicians indicated that the information should be passed on to family members, half of them thought that the decision to tell or not to tell should be left to the proband. In conclusion, while most of the surveyed physicians indicated that they would follow the principles of informed consent and autonomy, controversy exists regarding prenatal testing and termination of pregnancy.

Program Nr: 1211 from 2002 ASHG Annual Meeting

Importance of Telegenetics Services in India: A survey. *S.K. Pal, A. Kesari, G.S. Pandey, B. Mittal.* Medical Genetics, Sanjay Gandhi Postgraduate Ins, LUCKNOW, UP, India.

Communication and information have increasingly been considered important in helping patients to cope with chronic problems. Internet based health information can be shared with many people despite distance and time constraints. Videoconferences and data base facility are now effectively utilised for monitoring public health activity. High bandwidth services are creating a new way for patients and clinicians to interact and benefit from specialist services, which are available locally. Developing country has acute shortage of doctors and their services are concentrated in major cities, so in this situation telemedicine via Internet can play a crucial role in bridging this gap. A survey was conducted to assess the concept of telegenetic counseling by internet among 40 participants in a Genetic Counseling course in our department. The clinicians were from different states of India. A questionnaire was designed to know the general awareness regarding telemedicine and telegenetics. Centpercent of the participants agreed that the internet will play a major role in genetic counselling and patient's management at the peripheral level. Twenty(50%)of the participants were daily users of internet and 23% percent used it occasionally. Twenty eight (70%) thought that telegenetics can provide immediate accessibility of the experts and will greatly enhanced the scope of genetic services in the country. Thirty six(90%) of the clinicians agree to join the teleconsultant group and emphasized the idea of making an interactive websites in Indian context. All the participants wanted to be connected by net with the tertiary care hospitals after they completed the genetic counseling course.

Program Nr: 1212 from 2002 ASHG Annual Meeting

Tay Sachs Disease Screening, funding, publicity and community acceptance. *E. Sheffield, A. Gason, A. Bankier, M. Delatycki.* Genetic Health Services Victoria, Murdoch Childrens Research Institute, Melbourne, Australia.

The purpose of this study was to examine the roles of publicity, funding/sponsorship, education and other factors involved in bringing a genetic carrier screening program to a minority at risk group in a population, most of whom are unaware of the condition. The method used was that Tay Sachs Disease(TSD)screening was introduced to Jewish High School students in Melbourne in 1998. Funding for this program is limited. This program offers education to parents, students, and staff at 6 Jewish High schools. In 2000 it was extended to offer bi-annual community screening days to the Melbourne Jewish community. Financial sponsorship was provided from 1999 for a part-time genetic counsellor/educator, free testing for year 11 students, and subsidised testing for adults of child bearing age. Results showed that free testing of students increased the uptake from average 33%; (subsidised fee) to an average 67%; (free test and improved education program). Blood samples were used for tests. Surveys showed that a buccal smear would increase test uptake, and that students had a good knowledge about TSD following education sessions. In 2000, adults, of child bearing age, in the community were offered subsidised testing on community days. 15 people came to the first community day. An alternate publicity campaign increased this number to 70 in 2001. In conclusion, results indicate that it is possible to offer an effective and acceptable screening program on limited funding providing that it can tap into resources within the community and obtain the support of community leaders and relevant health professionals. The uptake of testing will increase if it is free; if it comes to the individual; if they understand why they are offered testing and if it is painless.

Impact of an Internet-based information service on the uptake of genetic services: the Orphanet experience. *S.M. Ayme, H. Nabarette, D. Oziel, S. Rastoul, B. Urbero.* INSERM SC11, Hopital Broussais, Paris, France.

The objectives were (1) to assess the influence of an Internet-based information service in the area of genetic diseases on the referral to appropriate health care services; (2) To compare the influence of this information on the patients and their relatives vs health care professionals; (3) To detect any harmful effect. Orphanet, a database of rare diseases and related services funded by the European Commission, was the internet resource facility which was evaluated. A survey was sent to the 500 clinics and the 350 laboratories which are referenced in the database and to 214 patients organizations. The survey aimed at assessing the impact of Orphanet on the number of referrals to specialized clinics and specialized laboratories. It included questions about the number of patients referred by an other professional and the number of patients having personally identified the clinics through Orphanet. It also included questions about the adequacy of referrals due to Orphanet. The response rate was 67% (144/214) for the support groups, 67,8% (460/678) for the clinics and 75,6% (229/303) for the laboratories. The clinicians are users of the website (80%), mainly to identify laboratories (60%), to refer a patient to an other specialist (52%), to find a support group (40%). The laboratories are also heavy users (76%). They look for other clinical (44%) or research (43%) laboratories. The support groups are users at 96.5%, mainly to refer patients to a specialized clinic. The assessment of the true impact is not yet finished but will be presented. This survey shows that the availability of information on highly specialized health resources may contribute to empowering consumers and to improve right use of these resources. The problems raised by such an information system are the validation of the information in areas where knowledge is scarce, the dissemination to the public of information which would be traditionally restricted to the professionals, the updating process, the legal issues, the criteria to classify places as expert centers and the extent to which some services can be delivered on line.

Iranian Human Mutation Gene Bank. *H. Najmabadi, M. Neishabury, F. Sahebjam, K. Kahrizi, N. Nikzat, M. Jalalvand, F. Aminy, S. Bani Hashemi, B. Moghimi, A.R. Noorian, A. Jannati, M. Mohammadi, M.K. Javan.* Genetics Research Center, The Social Welfare and Rehabilitation Sciences University, Tehran, Iran.

Completion of the Human Genome Project takes us to a new era in clinical genetics, where identifying the new genes responsible for the hereditary diseases. The existence of high genetic polymorphism within and between different communities in the world makes it necessary for the gene hunters to investigate many different populations. Iran, a large country with close to 66 million population, a land of different nationalities, tribes and religions offers a highly heterogeneous gene pool to the researcher in Genetics. The purity of many different races in this country has been greatly saved within the geographical borders and by an ancient culture that has always encouraged familial marriages. All these have created a remarkably heterogeneous population and yet high in consanguinity rate. During the last Five years investigation we have established a DNA bank (<http://www.IHMGB.com>) of all genetic diseases with Mendelian mode 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 undefined mutation. This bank stores patients and his/her first degree relatives DNA together with a comprehensive pedigree and clinical profiles for each sample. In order to facilitate collaboration with other scientists in the world with the same interests, we decided to present our experimental projects. This DNA bank providing opportunities for us to collaborate with outside, will offer a free of charge sample resource to all the research scientists in the world, , who are working on the various aspect on genetic disorders from prenatal diagnosis to gene structure and function. It is strongly stressed that no commercial benefit is involve in establishment of this DNA bank and the DNA samples are free of charge. However, to meet our goals and to respect the ethical values, DNA samples can only be used under certain conditions stated in the users consent form.

Newborn screening for treatable disorders in the Old Order Amish of Southwestern Ontario. *V.M. Siu^{1,2,3}, C.A. Rupa^{1,2,3}*. 1) London Health Sciences Ctr; 2) CPRI; 3) Departments of Pediatrics and Biochemistry, University of Western Ontario, London, ON.

The Old Order Amish in Southwestern Ontario represent a distinct, highly consanguineous population of about 2000 individuals. Their ancestors migrated directly from Europe to this area, and founding members are different from those of other colonies in the United States. In general, the Amish are quite knowledgeable about various diseases in their community, and keep excellent genealogical records. However, they tend to seek medical advice late in the course of illnesses, when treatment may be ineffective. One major factor contributing to the tendency to delay seeking help is the difficulty and expense of arranging for transportation, since the Amish usually utilize horse-and-buggy. Provision of medical care in their own community is likely to increase acceptance of medical intervention. Increased awareness of specific disorders and early identification of diseases allows targeted investigations and early treatment. Prenatal diagnosis of non-lethal disorders is generally not acceptable to the Amish, based on their religious beliefs. In this population, we have documented 5 disorders (PKU, galactosemia, cystinosis, cystic fibrosis, and juvenile glaucoma) amenable to treatment that may prevent or ameliorate morbidity if diagnosis is made at a presymptomatic stage. Previous work has identified the unique mutations for cystinosis and glaucoma in this group. Using a combination of molecular and biochemical tests, we will be initiating a neonatal screening program for the 5 treatable disorders. Discussions have taken place with Amish bishops and education sessions have been given to Amish women. The community has expressed its interest in early diagnosis and disease prevention. Support and ongoing education will be provided through public health nurses, local physicians, and a worker from within the community.

Survey of obstetrical providers' knowledge and attitudes regarding newborn screening. *S.M. Dolan¹, S. Gross¹, K. Damus², I. Merkatz².* 1) Division of Reproductive Genetics; 2) Department of Obstetrics & Gynecology and Women's Health, Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, NY.

Newborn screening is the first example of population-based genetic testing. State programs have grown since they were first developed in the 1960s, but advocacy for broader testing and advances in genetic technology have allowed for recent rapid expansion. Obstetricians have been called upon by newborn screening advocates to provide information about newborn screening to patients during pregnancy. **Objective:** The aim of this study was to assess obstetrical providers' knowledge and attitudes about newborn screening in order to provide resources to assist obstetricians. **Methods:** On a pilot basis, we developed a two-page survey which was distributed at Ob/Gyn Grand Rounds at our institution. **Results:** Thirty-nine surveys were completed and included in this analysis (34 MDs, 3 CNMs, and 2 PAs). Regarding knowledge about newborn screening, 59% of respondents said they knew "some" about newborn screening and 31% said they knew "very little." Only one physician reported knowing "a great deal" about newborn screening. When asked to list conditions that newborns are screened for in New York State, 23 respondents (59%) correctly listed phenylketonuria, 11 (28%) listed hypothyroidism, 8 (21%) listed HIV, 7 (18%) listed sickle cell disease, and 2 (5%) listed galactosemia. Thirty-four respondents (87%) stated that they would be interested in further education about newborn screening and most reported that Grand Rounds would be the best format. Regarding attitudes, in response to the statement, "Newborn screening is relevant to my practice," 4 respondents (10%) strongly agreed, 17 (44%) agreed, 16 (41%) stated they were not sure, and 2 (5%) disagreed. When asked if newborn is a topic that should be covered by the obstetrician, 21 (54%) stated they agree, 11 (28%) stated they are not sure and 7 (18%) stated they disagree. **Conclusions:** Increased education regarding newborn screening targeted to obstetricians and providers of obstetrical services can allow information regarding this important public health program to be disseminated to families and strengthen newborn screening programs.

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Experience with preconception screening for cystic fibrosis (CF) in a clinical molecular genetics laboratory. *K.G. Monaghan, D. Bluhm.* Department of Medical Genetics, Henry Ford Hospital, Detroit, MI.

October 1, 2001 the ACOG and ACMG recommended CF carrier screening be offered to all Caucasian couples who are either pregnant or planning a pregnancy and that CF carrier screening be made available to individuals of other ethnic groups. To educate physicians, we sent a summary of ACOG/ACMG's statement to all primary care physicians (including obstetricians/gynecologists, internists and family practitioners) within our institution and outside physicians who send specimens to us. This summary was also printed in our department's quarterly newsletter that is distributed to all physicians and doctoral-level scientists within our institution, outside referring physicians and reference laboratories from which we receive specimens. We included a one page brochure for patients describing CF, its frequency, inheritance, treatment and carrier testing. We have since experienced a 14.5-fold increase in the weekly sample volume for CF testing. The number of specimens received for carrier screening has increased greater than 17-fold and accounts for 61% of specimens received compared to 38% prior to October 1, 2001. Because of the large increase in sample volume, we changed our method of molecular CF testing. We previously used heteroduplex analysis and RFLP analysis to detect 55 CF mutations, which included the 25 recommended by ACOG/ACMG. We now use an oligonucleotide ligation assay (OLA)(Applied Biosystems) on a 3100 Genetic Analyzer to screen for 31 CF mutations and a combination of heteroduplex analysis and RFLP analysis to test for 12 additional mutations, 3 of which are recommended by ACOG/ACMG but not included in the OLA kit. The number of carriers identified is within the expected range. Among specimens received for general carrier screening, 26% were from couples in whom both partners were tested for CF, either concurrently or sequentially. Only one positive-positive couple has been identified; their fetus was negative for both parental mutations. We encourage other laboratories to educate physicians regarding CF preconception carrier screening and to be prepared for an increase in sample volume.

Barriers to genetic screening in the Ashkenazi Jewish population: The Philadelphia experience 1999-2002. *R. Keep*¹, *J. Becker*², *A. Schneider*¹. 1) Pediatrics, Albert Einstein Medical Center, Philadelphia, PA; 2) Department of Health Studies, Temple University, Philadelphia, PA.

An estimated 240,000 Ashkenazi Jewish (AJ) individuals live in the city of Philadelphia and the surrounding counties (Jewish Federation of Greater Philadelphia, 1997). If approximately 1 in 4 individuals of AJ ancestry is a carrier of one of ten diseases that occur at a higher frequency in this population, there would be an estimated 60,000 carriers in this region.

The AJ Genetic Disease Screening Program at Albert Einstein Medical Center was established in 1999 in response to the large Jewish population in this area, with funding provided by the Albert Einstein Society. Since its inception, the Program has provided outreach, education, genetic counseling and carrier screening to individuals of AJ descent. Despite extensive efforts to educate local rabbis, OB/GYNs and the public, via synagogues, Hillels, participation in Community events, publication of an educational brochure and annual free screenings, the overall interest and response from the Jewish Community has been disappointing.

Our experiences have led us to postulate that several barriers may be preventing AJ individuals from seeking genetic screening. These include:

- cost of testing and uncertainty regarding complete coverage by health insurance companies
- lack of understanding of recessive inheritance; that the absence of affected relatives does not eliminate the risk to be a carrier
- inadequate knowledge about these conditions and the availability of screening on the part of many OB/GYN physicians and rabbis
- fear of being identified as a carrier

A qualitative study was conducted using focus groups to explore the validity of our proposed barriers and to gain insight into the perspectives of different segments of the Jewish community regarding this issue. Results of these discussions will be presented. ••

Accountability in Diagnostic Molecular Genetic Testing: The U.K. *S.A.R. Stenhouse¹, S. Ramsden², S. Patton² and Steering Committee of UK NEQAS for Molecular Genetics.* 1) Institute of Human Genetics, Newcastle-upon-Tyne, U.K.; 2) National Genetics Reference Laboratory (Manchester), Manchester, U.K.

External quality assessment for molecular genetics has been operating in the United Kingdom since 1991. Beginning as an initiative of the U.K. Clinical Molecular Genetics Society the scheme became independent in 1994 and has expanded from offering three disorders to offering thirteen on a rotating basis. 43 laboratories from the U.K., Ireland and The Netherlands participate. The scheme aims to assess the whole analytical process with marks being awarded for genotyping, interpretation and clerical accuracy. Errors have been identified in all of these areas but mechanisms have been developed to identify poor performance and provide education and training for such laboratories. Over the years the scheme has seen an increase in conformity to consensus best practice guidelines and a corresponding reduction in errors leading to higher quality testing.

The development and structure of the scheme will be described in detail and comparison of the results over the years will be presented. Analysis of these results demonstrates that a robust system of external quality assessment contributes significantly to improving standards of molecular genetic testing. The accountability provided by participation in such schemes should give patients confidence in molecular genetic test results.

Program Nr: 1220 from 2002 ASHG Annual Meeting

A dynamic informed consent process for population based genetic research. *M.E. Smith, P.K. Manasco, T. Arledge.*
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Patient participation in population based research involving genetics is critical to achieving the goals of developing personalized medicines and understanding the role of genetic alterations in complex diseases. The current standard of practice for consenting study participants in most cases includes obtaining “blanket consent” from research subjects—consent for unspecified future use of biological samples and data generated from clinical trials in which the study participant agrees to “medical research” that may include future use of their DNA. The study participant may not be aware of these future uses. We agree with Beskow et al¹ that this approach may not allow study participants to make the most informed choices about participation in population based research involving genetics. We have developed a solution to the informed consent process that involves the use of an internet enabled dynamic informed consent process that allows study participants to control access and use of their phenotypic and genotypic information over time and throughout multiple studies by extending or restricting permission regarding the use of their previously collected biological samples and medical and genetic data. The study participant also has ongoing access to study information, is able to update personal clinical information and learn about new research studies. This environment provides online genetic education that is study specific and required for providing consent to participate in each research study, and also includes general genetic and ethical topics associated with research. We will review the various issues raised by individual Institutional Review Boards regarding the use of this new informed consent process for genetic research. These issues have included concerns about transmission of sensitive genetic information over the internet, how privacy is protected for individuals involved in studies utilizing the dynamic consent process, what is done with a study participant's genetic, clinical, and identifiable information at the end of a clinical trial, and how this consenting process is compliant with US HIPAA privacy regulations and the European Council and Parliament Directive 95/46/EC.

The ophthalmological window for identification of patients with Fabry disease. *M. Cybulla¹, B. Bausch¹, G. Franke¹, D. Schmidt², F.X. Glocker³, A. Gal⁴, K. Rosenberg⁵, H. Neumann¹.* 1) Dept. of Medicine, University of Freiburg, Freiburg, Germany; 2) Dept. of Ophthalmology, University of Freiburg, Freiburg, Germany; 3) Dept. of Neurology, University of Freiburg, Freiburg, Germany; 4) Dept. of Human Genetics, University of Hamburg; 5) Institute of Medical Biochemistry and Genetics, Copenhagen.

Background: Fabry disease is a rare X-linked disorder with galactosidase-A deficiency due to mutations of the AGAL-Gene resulting in globotriasylceramid deposits in various organs. Lifespan is reduced by about 20 years. Since a few months introduction of enzyme replacement therapy has revolutioned actual wellbeing and long time prognosis. Our estimations expect approximately 200 patients in Germany. Methods: All members of the German society of ophthalmology have been contacted by a questionnaire for patients with cornea verticillata. Such patients underwent pedigree analysis, biochemical analysis of serum galctosidase activity and investigation for classical skin lesions and dysfunction of th CNS, heart and kidney. Furthermore EDTA blood was drawn for genetic testing. Results: Response rate was 36% e.g. 1751 out of 4900 ophtalmologists returned the questionnaire. 24 patients with cornea vericillata where registrated. All had Fabry disease. So far, by clinical and genetic reevaluation the diagnosis was confirmed in 5 cases by clinical, biochemical, and genetic investigations. Conclusion: Nation-wide epidemiologic efforts using single components of rare diseases are effective for identification of such patients as shown for Fabry disease using cornea verticillata.

A focus group study of public understanding of genetic risk factors: The case of "a gene for heart disease". *B.R. Bates*¹, *A. Templeton*², *P.J. Achter*¹, *T.M. Harris*¹, *C.M. Condit*¹. 1) Speech Communication, Univ Georgia, Athens, GA; 2) Biology, Washington Univ, St. Louis, MO.

There is growing concern in the medical community about public perceptions of genetic determinism or genetic fatalism in the patient population. Limited information about how the public actually understands reports of genetic risk factors in disease formation is currently available. To understand public perceptions genetic risk factors a focus group methodology was employed to evaluate the phrase a gene for heart disease. There were a total of 108 participants recruited from urban, suburban, and rural communities in Georgia in July through October 2001. Participants were recruited to balance sex and racial representation. There were multiple interpretations of the phrase, but dominant interpretations did emerge. A gene for heart disease was interpreted as meaning both environmental and genetic risk factors played a role in disease formation. Genetic predisposition was perceived as heightened, not absolute, risk. The dominant perceived health impact of having the gene was a greater risk of becoming sick. Minority interpretations were found under each measure. There were no significant differences in expressed attitudes by sex. African-Americans expressed a higher level of perceived risk than did European-Americans. African-Americans were also more likely to perceive the medical effect of having a gene for heart disease as death or premature death, whereas European-Americans were more likely to perceive the impact as illness of premature illness. In the main, participants had medically correct evaluations of genetic risk factors and their implications for health behavior and treatment. The phrase a gene for heart disease does not appear to have the feared deterministic impact on participants perceptions of the level of risk from non-modifiable genetic risk factors. Concerns of genetic fatalism in patient populations may be overstated. Important considerations for provider intervention and patient education are indicated.

Genetic disease registries and informed consent. *B.A. Bernhardt, L.A. Hamby, G. Geller.* Johns Hopkins Medical Institutions, Baltimore, MD.

Genetic disease registries have been established or supported by many voluntary genetic organizations as a means to foster research on rare diseases. Possible risks of registry participation have been identified and include potential threats to privacy and the confidentiality of information. No guidelines exist on how informed consent should be obtained, nor on how researchers should gain access to registry data or participants. The goals of the current study were to explore how genetic organizations describe and use their genetic registries.

A two-page survey was sent to the executive directors of organizations listed with the Genetic Alliance (GA) as having a disease registry. Questions were asked about the purpose of their registry, perceptions of the risks of registry participation, whether informed consent is obtained from participants, and how researchers gain access to registry participants or data.

Of 173 organizations surveyed, 80 (46%) responded. Only 53(66.3%) respondents indicated that they currently maintain genetic registries, in spite of being listed as such with the GA. Forty-five organizations with registries (85%) mentioned research as a purpose and/or indicated that their registry has a mechanism by which researchers can obtain access to registry information. Of these 45, 33% indicated that they do not obtain informed consent before enrolling participants into their registry. Organizations that identify research as a purpose were significantly more likely to obtain informed consent ($p=.012$). However, there was no association between providing access to outside researchers and the likelihood of obtaining informed consent. When asked about the risks associated with being part of a genetic registry, 76% of organizations with a registry indicated that there were no risks to potential participants.

A substantial minority of organizations that maintain a registry for research purposes do not obtain informed consent. Most of these organizations do not identify any risks to registry participants. There is a need for developing and disseminating guidelines for informed consent for registry participation.

Ethical issues among a low-income minority population participating in genetic research. *N.H. Arar, V. Sartorio, R. Plaetke, H. Abboud.* Dept Medicine, Div Nephrology, Univ Texas Health Sci Ctr, San Antonio, TX.

We explored diabetic nephropathy probands and their relatives perspectives on: (1) revealing health, demographic and identifiable information about relatives without first obtaining their written consent and (2) their awareness of ethical issues and risks associated with their participation. Structured interviews were conducted with 246 Mexican American participants using Contextual Assessment Approach Questionnaire. 105 DN probands and 141 relatives were enrolled at UTHSCSA, SA, TX. Data analysis included both qualitative and quantitative methods using the software package SPSS. Average age of subjects was 56 years (range: 33-76, $s=9.04$); 62% were female. 72% of participants were not formally educated beyond high school and reported an annual household income less than \$20,000. 84% agreed to provide researchers with information on relatives age, gender and income. 65% of participants were willing to provide identifiable information such as names, addresses and phone numbers of relatives compared to 35% who were undecided. 61% of participants felt that there were direct benefits (i.e. supporting research) to disclosing information on relatives. 76% stated that there were no risks associated with disclosing information on relatives (i.e., discrimination) compared to 10% who felt that there were. While discussing potential risks, subjects did not consider these issues to influence their decision to participate in GFS. Many similarities between DN patients' and relatives' perspectives regarding revealing information about relatives and awareness of risks associated with their participation were identified ($P > 0.05$). One major ethical finding is the pervasive lack of awareness regarding risks associated with participation in GFS. Consequently, individuals who are unaware of their risks cannot provide informed consent when they participate in a study. We suggest that researchers identify better ways to ensure voluntary and informed participation while conducting GFS among a low-income minority populations. This work has been funded by ELSI/NIH and an IRG from UTHSCSA. The ascertainment of families has been funded by NIDDK/NIH.

African-American Views on Ethics in Genetics. *J.C. Fletcher*¹, *D.C. Wertz*². 1) Keswick, VA; 2) University of Massachusetts Medical School, Shriver Div., Waltham, MA.

To study Americans' views on ethical issues in genetics, we surveyed 1000 members of the adult public, using anonymous questionnaires administered door-to-door by a professional survey firm. 988 (99%) responded, including 111 (11%) African-Americans, whose responses differed significantly ($p < .05$) from the total group on 26 (52%) of 50 ethical questions. More African-Americans than the total group showed concern about the needs and rights of blood relatives and spouses/partners, wanted professionals to make direct suggestions, and said don't know in response to questions about alternative reproductive technologies or PND for sex selection. Fewer would abort for genetic conditions or would support adoptees' rights to medical information about birth parents. 32% of African-Americans (compared with 44% of the total) thought professionals should preserve confidentiality of a patient who refused to share genetic information with relatives at risk; 37% (compared with 27%) thought professionals should tell a woman's partner about non-paternity; 33% (compared with 22%) supported involuntary hospitalization for a PKU mother who cannot stay a special diet; 36% (versus 43%) thought employers should have no access to genetic information, even with consent; 36% (versus 47% of the total) would abort if the child would be severely retarded unable to speak or understand; 30% (versus 41%) would abort if the child would be paralyzed from the neck down; 41% (versus 48%) would abort if the child would die soon after birth; 14% (versus 22%) would abort if the child would be moderately retarded (able to speak, but not live independently). African-Americans did not differ from the total in regard to individual confidentiality versus public safety, reports of refusals of employment or insurance on genetic grounds, genetic testing of children, preconception sex selection, genetic testing in the workplace, and legality of abortion after PND. Results indicate a need for further study of views of minority groups.

Effects of non-disease genetic information on the self-concept of individuals in the FAMuSS study. *E.S. Gordon¹, J.M. Devaney¹, B.T. Harmon¹, M.J. Hubal², P.M. Clarkson², E.E. Pistilli³, P.M. Gordon³, E.P. Hoffman¹.* 1) Ctr for Genetic Medicine, Children's Natl Med Ctr, Washington, DC; 2) University of Massachusetts, Amherst, MA; 3) West Virginia University, Morgantown, WV.

The use of SNPs in genetic testing and research is a rapidly expanding field. Already companies are offering genetic testing for non-disease traits. Before forging forward, we must consider the potential psychological impact of collecting and disseminating non-disease genetic information. Here we examine change in self-concept related to disclosure of non-disease genetic information regarding the ability to gain strength and muscle mass. We present preliminary data from the first 49 subjects of a 1400 subject, 4-year exercise/genotyping study at 7 universities (Factors Affecting Muscle Size and Strength; FAMuSS study). To begin to address the issue of "genetic self-knowledge" and self-concept, we asked subjects to enroll in a genetic disclosure and self-concept/ethics arm of the FAMuSS study. At the time of enrollment (T1), subjects completed a series of self-concept evaluations including: Tennessee Self-Concept Scale; Health Orientation Scale; and supplemental questions on knowledge of genetics designed for this study. Each subject donated a blood sample for genotyping of 4 SNPs previously shown to influence muscle performance: ACE, CNTF, g-Sarcoglycan, and UPC-2 genes. After completing a 12 week exercise program, we told subjects which SNPs they possess and we gave information about the traits associated with each SNP. The self-concept evaluation was then repeated (T2). 14% of subjects felt that the genetic information they received changed their self-concept and 32.6% said that the information would change their behavior. Comparing the perceived ability to gain strength and muscle mass, between T1 and T2, 18.4% perceived some change in ability to gain strength and 24.5% perceived some change in ability to gain muscle mass. These results indicate that non-disease genetic information has the potential to alter both self-concept and behavior. The effects of receiving non-disease genetic information should be investigated further before commercial genetic testing expands to non-disease factors.

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Multiplex Heterozygote Screening for Autosomal Recessive Conditions in the Ashkenazi Jewish Community An 8-Year Experience. *V.M. Jansen, L. Zajac, E. Reich, J. Borsuk, L. U, M. Bannerji, J. Li, H. Ostrer, C. Oddoux.* Human Genetics Program, New York Univ School of Medicine, New York, NY.

Since 1975, the Human Genetics Program at New York University School of Medicine has provided heterozygote screening for members of the Ashkenazi Jewish community. Over 15,000 people have been tested. Initially, only testing for Tay Sachs disease was offered, but over the last 8 years, other tests have been added, including: cystic fibrosis (CF), Canavan disease (CD), Gaucher disease (GD), Bloom syndrome (BS), Fanconi anemia (FA), Niemann-Pick disease (NP) and familial dysautonomia (FD). The willingness of patients to participate in research trials has led to the ascertainment of allele frequencies and thus to the contemporary testing strategy (TS 1:25, CF 1:25, CD 1:41, GD 1:7, BS 1:107, FA 1:80, NP 1:90, FD 1:36). Collectively, the likelihood for an individual to be a carrier for at least one of these conditions is 30%. With education, currently via web-based video and genetic counseling, patients have made informed decisions regarding screening with 2/3 choosing multiplex testing, the majority of whom have elected to be tested for all 8 disorders. Many patients have returned to update their testing with subsequent pregnancies. All carrier couples identified through the program have opted for prenatal diagnosis. The identification of the IKAP gene for familial dysautonomia in 2001 led to a demand among the relatives of affected individuals for carrier testing. One of the most difficult outcomes of screening has been the identification of previously unknown homozygotes for Gaucher disease. Although these tests have been used principally for heterozygote detection, their availability has led to requests for diagnosis of suspected homozygotes/compound heterozygotes. The identification of prevalent founder mutations for mucopolysaccharidosis IV, familial hyperinsulinemia, glycogen storage disease Type I, lipoamide dehydrogenase deficiency, alpha-1-antitrypsin deficiency, and Usher syndrome suggests that screening for these conditions might also be important.

Living with BRCA1/BRCA2 test results: Women's social experiences. *I. Nippert¹, B. Teige¹, J. Horst².* 1) Women's Health Research, Universitaetsklinikum Muenster, Muenster, Germany; 2) Institut fuer Humangenetik, Universitaetsklinikum, Muenster, Germany.

Introduction: Since the introduction of BRCA1/BRCA2 tests into routine medical care in the late 1990's, more and more women at increased risk undergo predictive genetic testing. Little is known about women's long term social experiences. **Methods:** At the Department of Human Genetics, Universitaetsklinikum Muenster, Germany, women at increased risk are offered BRCA1/BRCA2 testing. Pre- and post-test counseling is mandatory. Out of 50 women who underwent testing the last 2 years and who have obtained test results at least 6 months ago were asked to participate in a follow-up interview. 46 women agreed to participate. A standardized questionnaire addressing questions such as: communicating risks to family members; conflicts with family members because of test results; positive and negative experiences; decision regret; availability of preventive surveillance; etc. was administered by telephone interview. **Results:** 58.7% report that their main reason to undergo testing was to know more about their personal risk; 60.1% wanted their children to have certainty about a hereditary risk; 23.9% wanted to know their risk in order to optimize personal future plans; 58.7% reported that their expectations were fully met. 23.9% reported that they only underwent testing because other family members asked them; 52.2% wanted to optimize prevention. More than 98% consulted with family members and all informed, albeit, selectively other family members about the result. 13% expressed decision regret and 87% would undergo testing again, 60.9% would recommend the test to other women at increased risk. **Discussion:** Few women who undergo predictive BRCA1/BRCA2 testing after a pre- and post-test counseling protocol express decision regret after a ≥ 6 month period.

The effect of gene patents on the provision of clinical services: a comparison of laboratory directors' and genetic counselors' practices. *M.A. Weaver¹, M.H. Henry², M.K. Cho¹, D. Leonard², S. Parthasarathy³, C.L. Bosk², J.H. Barton¹, J.F. Merz².* 1) Stanford University; 2) University of Pennsylvania; 3) Duke University.

There is growing concern about the impact of gene patents on the provision of clinical services. This research addresses laboratory directors' and genetic counselors' experiences, as the providers of clinical services, with 8 genetic diseases and provides new data on the extent to which patenting and licensing of disease genes are affecting the provision of DNA-based genetic testing. The two main research questions addressed are: 1) How are laboratories that perform DNA-based genetic tests affected by patents and licenses, and 2) What do genetic counselors perceive the impact of gene patents and licenses to be on the provision of clinical services. We sampled 118 U.S. laboratory directors who were members of the Association for Molecular Pathology (in 1998) or who were listed on GeneTests on 6/18/01. We also selected a random sample of 200 genetic counselors who have been counseling for three or more years and are full members of the National Society for Genetic Counselors. We conducted, taped, and transcribed semi-structured telephone surveys of roughly 30 minutes each. Preliminary findings from interviews with 45 laboratory directors and 63 genetic counselors show a wide range of familiarity with gene patents. Laboratory directors demonstrate a greater awareness of gene patents than do genetic counselors. When asked specifically about the 8 particular tests, laboratory directors indicated that the patents affected their ability to provide genetic testing in a variety of ways, including discontinuation of or a decision not to adopt a test, cost increases, and a need to develop alternative testing methods. In contrast, genetic counselors did not report a change in their counseling methods or a notable change in test quality or cost due to the existence of a patent. However, most genetic counselors reported that insurance coverage is currently the main obstacle to genetic testing. These findings suggest that gene patents have a greater impact on laboratory directors than on genetic counselors.

The significance of identical triplet repeat numbers for both X chromosomes in developmentally delayed/mentally retarded females negative for a FMR-1 mutation. *L.R. Shapiro^{1,2,3}, R.F. Walsh¹, R. Scott-Oresajo³*. 1) Department of Pediatrics, Westchester Medical Center, Valhalla, NY; 2) New York Medical College, Valhalla, NY; 3) Regional Medical Genetics Center, Hawthorne, NY.

Direct DNA analysis of the FMR-1 gene in developmentally delayed/mentally retarded children resulted in 237 (140 males; 97 females) who were negative for a Fragile X mutation. In 28% of the females (27/97), an identical number of CGG triplet repeats was found for each X Chromosome. In order to determine the significance of this observation and the probability that each of the mothers and fathers have identical CGG triplet repeat sizes, the triplet repeat size of each of the 237 patients was determined and the distribution frequency of each triplet repeat size was compared to the reports in the literature. The distribution of the CGG triplet repeats in this cohort was the same as reported in the literature with 29, 30 and 31 as the most prevalent triplet repeat size.

Direct DNA mutation analysis of 3 sets of parents whose daughters had the common 29, 30 or 31 CGG triplet repeat size on both of their X chromosomes revealed that both parents of each set had identical triplet repeat sizes. In 2/27 females, the infrequent 20,20 and 24,24 CGG repeats were found. In order to attempt to determine the significance of this observation and to determine if Uniparental Disomy X (UPD X) was present, direct DNA analysis of the FMR-1 gene of each of the parents revealed that the mother and father each possessed identical CGG triplet repeat numbers.

Thus, it can be concluded that in these families, UPD X was not present or responsible for their daughters' clinical status; however, additional cases will be necessary to further determine the parent of origin of infrequently occurring identical CGG triplet repeat numbers. It is possible that parents with the same infrequent CGG triplet repeat number may share other genetic similarities which could predispose to autosomal recessive etiologies of non-specific mental retardation in females.

Use of a birth defects registry to assess physician utilization of FISH analysis in children with cardiac defects.

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Birth Defects registries have typically been used to determine the prevalence of birth defects and to identify regional clusters of birth defects. They have not often been used to evaluate utilization of cytogenetic testing. We determined that the Oklahoma Birth Defects Registry (OBDR) could be used to assess the utilization of fluorescent in situ hybridization (FISH) analysis for children with cardiac defects. Congenital heart defects (CHD) are the most common malformations associated with the chromosome 22q11.2 deletion. Patients with this deletion may have other abnormalities including immunodeficiency, hypocalcemic hypoparathyroidism, palate defects, renal defects, hypotonia, developmental delay and psychiatric problems. An accurate diagnosis by FISH analysis is essential to provide appropriate clinical management, specific interventions, and recurrence risk counseling. All cases listed in the OBDR with tetralogy of Fallot, interrupted aortic arch, truncus arteriosus, double outlet right ventricle, aortopulmonary window, and vascular ring from January 1999 through December 2001 were reviewed to identify the hospital where the cardiac defect was diagnosed, the age at diagnosis, whether chromosome and FISH analysis were performed, and demographic data. Of the 144 cases identified, 10.4% (15 cases) had FISH testing, 29.9% had chromosome analysis without FISH testing, and 59.7% had no documentation of chromosome or FISH testing. FISH analysis identified the 22q11.2 deletion in 60% (9 cases). Tertiary hospitals in Oklahoma City diagnosed 63% of the cases whereas those in Tulsa diagnosed 37%. One-third of the FISH testing was ordered by Oklahoma City hospitals and two-thirds was ordered by Tulsa hospitals. The majority of infants (83.3%) were diagnosed at birth before discharge. The information from the OBDR is being used to follow-up patients and to develop educational materials for physicians and other health professionals about the appropriate utilization of FISH analysis for children with CHD. The OBDR will also be used to measure the effectiveness of educational programs.

Subtelomeric FISH analysis: Recent experience and referral trends in a clinical cytogenetics lab. *P.A. Ward, L.G. Shaffer, R. Saleki, C.A. Bacino, B. Bejjani, R. Naeem.* Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX.

We report our experience over the past 28 months (Jan 2000 - April 2002) using subtelomeric region-specific FISH probes (CytocellTM) to examine a total of 655 patients. The majority of patients (>97%) were referred for evaluation of unexplained mental retardation (MR) or developmental delay (DD). Referral sources included genetics professionals (64.1%); other physician specialists (21.5%); referral or cytogenetics labs (14.4%). A significant increase in referrals from other physician specialists was observed during the past 14 months. G-band analysis was previously or simultaneously performed on most patients, however these data were not provided on all patient samples submitted for testing.

Clinically significant rearrangements of subtelomeric regions were identified in 28 patients (4.3%). Seventy-five percent of these patients (21/28) was referred by a genetics professional. A variant or probable variant was identified in 49 patients (7.5%). Of these, 45 cases involved an apparent deletion of 2q37.3. No subtelomeric rearrangements were identified in the remaining 578 patients (88.2%). Parents for 5 of the 28 (17.9%) abnormal cases were studied and 3 parental balanced translocations were identified. Parents of 13 of the 49 (26.5%) variant cases were studied and all were found in one unaffected parent (10 were 2qter deletions).

These data support the use of subtelomeric region-specific FISH to fully evaluate patients with MR/DD. Our experience is consistent with recent reports showing subtelomeric alterations in 3-7% of such patients. Our data also confirm the identification of variants in >7% of individuals, particularly the 2q37.3 deletion. These test outcomes suggest that genetic counseling prior to telomere FISH analysis should include discussion of the significant likelihood (10-15%) that parental analysis will be recommended to clarify the significance of their child's results. The integration of genetic counseling and phenotypic evaluation will improve and guide future test utilization.

A novel approach for detection of DAZ gene copy number in male infertility. *A. Rossi, A. Tessari, E. Salata, L. Bartoloni, M. Simonato, G. Rizzo, A. Ferlin.* Medical and Surgical Sciences, University of Padova, Padova, Italy.

Introduction. The DAZ gene family constitutes the major candidate for the AZFc (azoospermia factor c) phenotype of male infertility, being deleted in about 10% of azoospermic and severely oligozoospermic subjects. Four nearly identical DAZ genes are arranged in two clusters in AZFc, and standard analysis by PCR cannot distinguish among the different copies. In fact, this analysis can detect only deletions removing the whole DAZ gene cluster. However, it can be assumed that the frequency of DAZ gene abnormalities in infertile men might even be underestimated. **Materials and methods.** By using particular sequence tagged sites (STSs) and restriction enzymes it is possible to differentiate the individual DAZ genes. This approach is based on subtle sequence differences, termed sequence family variants (SFVs), among members of the DAZ genes. We developed a PCR amplification-restriction digestion assay able to distinguish DAZ genes for three SFVs. PCR products were digested with *Sau3A* (sY581), *DraI* (sY587) and *TaqI* (sY586) and the obtained fragments were run on a 12% acrilamide-bisacrilamide gel electrophoresis and visualized by silver staining method. Direct sequencing on both strand was also performed for these PCR products and for another one (DAZ SNVII). We applied this approach to screen a group of 30 fertile men and 80 idiopathic severely infertile men (sperm count < 5 million/mL). **Results and discussion.** Combining the data obtained by these PCR-restriction digestions and sequencing, 6 patients out of 80 (7.5%) showed partial deletion of the DAZ gene clusters. These partial deletions were also confirmed by Real Time PCR using a specific TaqMan probe on ABI PRISM 7000 Sequence Detection System. Different deletions of DAZ genes may be present and can cause a severe spermatogenic damage. This preliminary screening demonstrate that deletions of copies of DAZ genes may be found with high frequency in severely infertile men and strengthens the role of this gene family in spermatogenesis. Furthermore, these methods could be used to screen a larger number of patients and to perform a more accurate diagnosis.

Quantitative assay for the detection of *Streptococcus* DNA in mouthwash samples. *P.K. Bender, W.R. Beggs, J.C. Beck.* Coriell Cell Repositories, Coriell Inst Medical Research, Camden, NJ.

The collection of biological samples from mouthwash is becoming an increasingly popular method as a source of DNA in large epidemiological studies. Mouthwash samples have the advantage of being cost effective, non-invasive, promote subject compliance, and provide a source of DNA that can be genetically analyzed. However, the analysis of DNA from mouthwash samples is complicated by the presence of extraneous DNA sources from oral bacteria and food particles, and suffers from a high variability in the amount of DNA obtained. The reason for the high variability is not understood. It may arise from phenotypic differences between individuals, behavioral differences, or from differences in the amount of bacteria and food particles that are obtained. In order to investigate the contribution of bacterial DNA in mouthwash samples, an assay was developed to measure *Streptococcus* DNA. The assay is based on the 5 fluorogenic real time method using primers and a fluorogenic probe targeting the amplification and detection of a 16S rDNA sequence. This sequence is shared among the four common *Streptococcus* species found in the oral cavity mutans, oralis, salivarius, and pneumonia. The assay does not detect human DNA or *E. coli* DNA. Using a standard of purified DNA from a culture of *Streptococcus mutans*, as little as 16 picograms of DNA can be measured even in the presence of excess human DNA. The method has been applied to the analysis of mouthwash samples collected in a pilot study that found the yield of total DNA to be significantly greater from samples collected under unsupervised conditions (at home) versus supervised conditions. The question arises as to whether unsupervised samples have higher amounts of bacterial DNA. However, measurements with this 5 fluorogenic assay reveal that although the amount of bacterial DNA present is highly variable, there is no significant difference between the unsupervised and supervised samples. Thus, the difference in total DNA yield between the two collection methods likely occurs from differences in human DNA. Further studies with mouthwash samples from larger collections are ongoing.

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ABCC7 S158N (605G>A) is a rare genetic variant found in coupling with DF508 in a cystic fibrosis family: why parents should also be genotyped. *A.E. Shrimpton, K.E. Hicks, W.V. Beadling.* Clinical Pathology, SUNY Upstate Medical Univ, Syracuse, NY.

The Molecular Diagnostics Laboratory at SUNY Upstate Medical University uses a home-brew 35 cystic fibrosis (CF) mutation detection method that includes an Mse I restriction digest of exon 4 amplicons. An unusual restriction fragment pattern was observed in a sibling of an individual with CF. A single nucleotide change, 605G>A, that causes a novel Serine (Ser) to Asparagine(Asn) change at codon 158 (S158N) in exon 4 of the CFTR gene, ABCC7, was detected in this individual, who was also heterozygous for delta F508. Family studies revealed that the CF affected sibling also had both mutations, however S158N was in coupling with delta F508, both having been inherited from the same parent. The mutation from the second parent has not yet been identified. The only other report in the CF mutation database involving this conserved codon was the report of S158R (604A>C) in a CBAVD patient. ABCC7 intragenic and extragenic markers confirmed that the original patient was a CF carrier. S158N is thus a rare genetic variant, of unknown clinical significance, in coupling with delta F508 in this family. Only by investigating the parents could phase be established. Relying on the CF affected patient's genotype alone may have lead to the incorrect conclusion that this individual also had CF molecularly. Whenever possible parents should also be genotyped so as to confirm phase and reduce the chance of mistyping from double mutants, deletions or amplification drop out.

New DNA Capillary Array for mRNA Expression Profiling. *Y. Hatta-Ohashi¹, E. Shinohara¹, T. Takahashi¹, K. Hashido¹, N. Morimoto¹, T. Makino², S. Karki¹, K. Tokunaga³.* 1) Genome Medical Business Division, Olympus Optical Co.,Ltd., Tokyo, Japan; 2) NovusGene Inc, Tokyo, Japan; 3) Department of Human Genetics, Graduate School of Medicine, University of Tokyo, Tokyo, Japan.

DNA microarray technology based on a highly parallel DNA hybridization assay is widely used for gene expression profiling and disease diagnostics. In the present study, a new gene-detection device was developed using a modified type of DNA microarray, which can operate samples as little as 3-5 microliters. This new DNA microarray system is characterized by 8 or 20 capillaries formed on the silicone rubber (polydimethylsiloxane), 100 microns in depth and 0.5 or 1.0 mm in width. In each capillary, 50 to 100 oligonucleotide probes can be immobilized. The use of capillaries enables us to test multiple samples simultaneously for examining expression levels of selected genes rather than those of numerous ones. The oligonucleotide probes to be attached on the capillary are designed by our new algorithm based on the tuple method. A tuple is a short sequence consisting of several bases. Each tuple contained in a candidate sequence for the probe is evaluated for its uniqueness in the all registered sequences of genes and ESTs in public databases. A sequence which contains a "frequent" tuple is not selected as a probe, allowing us to find a probe with high specificity. Our software with this algorithm consists of further several calculation filters for the determination of an optimal sequence that has an appropriate melting temperature and does not form an intramolecular structure. In order to evaluate a reproducibility of the DNA capillary array, mRNA expression levels of 60 genes were compared between two capillaries on the same substrate, using labeled mRNA samples from Jurkat T cells. The result showed a high correlation between the two capillaries ($R\text{-square}=0.97$). This new DNA capillary array is relatively inexpensive, because a semiconductor substrate is not necessary and regular glass can be used. Detailed comparisons of our method with other conventional ones will be described. (This work was supported by NEDO [New Energy and Industrial Technology Development Organization] in Japan.).

Diagnosis of haploinsufficiency and duplication of SHOX using a real-time PCR. *E.J. Seo^{1,2}, M.J. Park¹, S.M. Park¹, Y.O. Yoo¹, Y.L. Shin^{1,3}, H.W. Yoo^{1,3}.* 1) Genome Research Center for Birth defects and Genetic disorders, Asan Medical Center, Seoul, Korea; 2) Dept. of Clinical Pathology, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea; 3) Dept. of Pediatrics, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea.

Short stature homeobox-containing gene (SHOX) is on the pseudoautosomal region of the sex chromosomes and plays a role in bone growth and development. SHOX escapes X inactivation and relates to a gene dosage effect. Haploinsufficiency of SHOX is the cause of short stature in Turner syndrome, and has also been discovered in Leri-Weill dyschondrosteosis and idiopathic short stature. On the contrary, overdosage of SHOX usually leads to tall stature and gonadal dysgenesis. Current methods to determine gene dosage are time-consuming and labor-intensive. We present a rapid and accurate method to assess gene copy number for detection of haploinsufficiency and duplication of SHOX based on a real-time quantitative PCR. We studied 10 patients with Turner syndrome containing a karyotype of 45,X or Xp deletion, 12 patients with a karyotype of 47,XXY or 47,XXX, 45 patients with idiopathic short stature, and 40 normal control individuals. By using TaqMan probes specific to SHOX and PBGD genes as a reference gene, quantification of gene copy number was analyzed through the comparative CT method. The observed numbers of the SHOX gene per haploid genome were 0.940.14SD in 40 normal controls, 0.500.13SD in patients with Turner syndrome, 1.550.11SD in patients with extra sex chromosomes, respectively. One of 45 patients with idiopathic short stature showed a haploid copy number of approximately 0.5. Therefore, the dosage test using real-time PCR could be easily and accurately applied to the diagnosis of diseases caused by changes in gene dosage.

Comparison of SNP Detection Methods and Instrumentation Platforms. *C.M. Strom¹, W. Sun¹, M. Zoleikhaeian¹, C. Li², Y. Naidu², G. Milburn³, R. Fenwick⁴, M. York¹, T. Entwistle¹.* 1) Molec Gen, Quest Diagnostics, San Juan, Capist, CA; 2) Center for Advanced Genomic Technology, Quest Diagnostics, San Juan, Capist, CA; 3) Molecular Diagnostics and Virology Laboratories, American Medical Laboratories, Chantilly, VA; 4) Molec Endocrinology, Quest Diagnostics, San Juan, Capist, CA.

Accurate, robust, cost-effective and high throughput single nucleotide polymorphism (SNP) genotyping is essential for clinical diagnostics. Using detection of C282Y and H63D mutations in the HFE gene as a model system, we have evaluated several common methodologies and instrumentation platforms: allele-specific oligonucleotide (ASO) hybridization with chemiluminescent detection, 5-nuclease (TaqMan) assay with ABI7700 detection, MassExtend (Sequenom) assay with MALDI-TOF mass spectrometry, electronic hybridization on the NanoChip workstation, Pyrosequencing technology on PSQ96 system, fluorescent restriction fragment length polymorphism (RFLP) using ABI3100 genetic analyzer and PCR-ASO using the mDx reagents from BioRad. Over 1000 genomic DNA samples were analyzed using each of the methodologies and/or platforms listed above. Genotype results were compared among the methods and the discrepancies resolved through repeat testing and / or sequence analysis. Our findings in terms of assay accuracy, robustness, cost, and user-friendliness of the SNP detection platforms will be discussed.

An efficient application of MALDI-TOF/MS coupled with microarray for detection of microsatellite

polymorphisms. *Y. Yoshikawa*^{1,2}, *K. Nakajima*¹, *N. Kimura*², *M. Gonda*³, *K. Okamoto*¹, *G. Tamiya*¹, *H. Inoko*¹. 1) Sch Med, Tokai Univ, Isehara, Kanagawa, Japan; 2) Nisshinbo Industries, Inc. 1-2-3 Onodai, Midori-ku, Chiba, Japan; 3) Shimadzu Corporation, 1, Nishinokyo-Kuwabaracho, Nakagyo-ku, Kyoto, Japan.

We have investigated a genotyping method for microsatellite polymorphisms using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) in combination with a microarray format. MALDI-TOF/MS offers unprecedented speed for analysis with excellent accuracy. However, the conditions for detection of large DNA fragments that is needed for the detection of microsatellites have not yet been determined. In order to optimize the conditions for the detection of large DNA fragments, we first examined matrices, small organic molecules, which are a key part of mass spectral methods. We have screened 147 compounds as potential matrices, and found several useful compounds for the analysis. Among them, the best results were obtained with 2,4-dehydroxyacetophenone. With this matrix, we successfully detected the combination of an 100-mer and 102-mer TC repeat nucleotides precisely. To overcome the problem of fragmentation of purine sequences, we have explored the effects of purine analogs on the stability of the oligomers. We have found the utilization of 7-deaza purine analogues allowed excellent detection with an improvement in sensitivity and a reduction in oligomer fragmentation. Next, we examined a microarray-based approach using carbodiimide-coated glass plates instead of expensive stainless-steel plates that are commonly used in MALDI-TOF/MS analysis. As a result, we detected DNA fragments on glass plates with extremely high-resolution that was sufficient to distinguish single-nucleotide differences. Furthermore, we developed an oligomer method to hybridize with the target DNA fragments on the glass plates for their final detection by MALDI-TOF/MS without any non-specific signals. These results indicate that MALDI-TOF/MS coupled with microarray technology is quickly emerging as a powerful approach for determination of polymorphic microsatellites.

Mutation analysis of Rett syndrome patients. *M. Ito, J.M. Milunsky, A. Milunsky.* Center for Human Genetics and Department of Pediatrics, Boston University School of Medicine, Boston, MA.

We have analyzed over 600 samples sent to the DNA Diagnostic Laboratory for Rett syndrome testing by mutation analysis of MECP2 gene, in which at least 140 patients are believed to exhibit classic signs of this disorder. To date we have identified 120 positive probands among them, in which the first 65 positive patients (out of approximately 300 analyzed) were reported in Milunsky et al., 2001 (*Genetic Testing* vol.5;321-325). Here, we report 55 additional patients (out of approximately 300 analyzed). The current five common mutation screen (T158M, R168X, R255X, R294X and R306C/H) detects only 33% of all patients that we identified as positives. In the past, however, the detection rate for these five mutations was as high as 50% (Milunsky et al.). The remaining positives were identified by bidirectional sequencing. All positives were females with no family history. One plausible reason for recent reduction in the detection rate of these five mutations would be a change in submitted patients' clinical features. In the past, we had a higher proportion of patients with classic clinical manifestations who had any one of these five mutations, however, currently we have a higher number of patients with atypical clinical presentations who have mutations other than these five. We have identified nine novel mutations; three point mutations (N126S, G238R and H368Y), five deletion mutations (495delC, 816delG, 995del258bp and 1163del39bp) and one insertion (902ins4bp). In addition, we have identified two novel polymorphisms and three novel possible polymorphisms (G161V, A357T and A378G). In these two novel polymorphisms, we have detected the same nucleotide change in the mother with random X inactivation in one case (P388L), and we found the same nucleotide in the father in the other case (P376S). Except for deletions and insertions that cause frame shift and point mutations that lead to nonsense mutations, we recommend all other novel base substitutions be tested in both parents to identify if the change is pathologic. In addition, if the mother has the same nucleotide substitution, a skewed X inactivation study should be performed.

A comprehensive method for GBA whole gene-sequencing with a rapid first step screening of known Gaucher Disease mutations. *M. Keddache, G. Grabowski.* The Children's Hospital Research Foundation, Division of Human Genetics, Cincinnati, OH.

Gaucher Disease, a common autosomal recessive glycosphingolipidosis, is characterized by hematologic abnormalities with hypersplenism, hepatomegaly, bone lesions, and the presence/absence of CNS disease. Certain mutations in the GBA gene reduce the levels of activity of acid-beta glucosidase, leading to the manifestation of the disease. But the genotype-phenotype (disease severity) is not well correlated, suggesting the existence of modifier mutations possibly within the GBA gene itself. A two-phase procedure was developed for the determination of GBA variants related to Gaucher Disease. The interrogation phase provides a rapid answer about known mutations and is appropriate for testing disease allele transmission to offspring of Gaucher patients, pre- or post-natally. The discovery phase is more time intensive, but provides a comprehensive answer about the sequence variations at GBA for any sample. A single ~ 7.5 kb PCR product containing the entire GBA gene is used as a template for both the interrogation and discovery phases. In the interrogation phase, 8 common mutations are tested for simultaneously using single nucleotide extension technology. The panel is being expanded to include additional interrogatable base changes. From receipt of the test sample, all SNPs in the panel are accessible within 48 hours. The discovery phase takes place when more information is needed about the patient's genotype than just the presence of the mutations in the interrogation panel. Complete sequencing of GBA is accomplished using the ~7.5 kb template and a set of 16 primers. The sequences are assembled into a single contig and the traces are visually inspected to confirm heterozygote base calls. Completion of the discovery phase is achieved within 72 hours. After screening 70 alleles from a cohort of Gaucher patients and relatives, 6 previously unreported polymorphisms were discovered. With additional data, a better understanding will be obtained for the contribution of silent or intronic mutation to the Gaucher phenotype in conjunction with mutations causing reduced enzymatic activity.

Is DG heterozygosity clinically significant? Literature review and analysis. *V.K. Proud¹, M.L. Lawson², M.A. Barnes², H.A. Creswick¹*. 1) Medical Genetics, Dept Pediat, CHKD/EVMS, Norfolk, VA; 2) Clinical Outcomes Research and Epidemiology, Dept Pediat, CHKD, Norfolk, VA.

Most infants found in newborn screening for galactosemia are simple heterozygotes. In Virginia (1998) out of 92,000 infants, 40 had abnormal tests, 6 had classic galactosemia (GG) with minimal galactose-1-phosphate uridylyltransferase (GALT) activity; while 34 had variants like Duarte/galactosemia (DG) with $\gg 25\%$ GALT. DG heterozygotes should be at minimal risk for complications, however, many are treated for the first year, and girls are more frequently treated than boys. We performed a comprehensive literature review and analysis to determine whether DG heterozygotes have medical complications. From 701 articles from Pub Med (1965-2002) using search strategy including "galactosemia OR Duarte OR genotype OR phenotype" and restricted to Human and English, 33 articles met inclusion criteria. There were 8 case series, 9 case-control, 11 cohort, and 5 cross-sectional studies. There was only one 35 year cohort study from Germany addressing outcome. Regarding complications in DG heterozygotes: 12/33 authors reported complications; 10/33 completely excluded complications; 7/33 neither supported nor denied and 4/33 both supported and denied clinical complications. In the 19 articles that focused on DG heterozygotes, 7/19 supported clinical findings and 4 had case reports of cataracts, liver dysfunction, or decreased ovarian function. One study reported increased frequency of Duarte alleles in families with a history of ovarian cancer. Biochemical studies predicted mixed results depending on parameters studied. Finally, in 7/19 there was data that clearly denied any abnormal phenotype in DG individuals. In conclusion, although rare case reports and some biochemical data suggest potential for long term medical complications in DG heterozygotes, there is no consistent outcome reported in the current literature. This study strongly supports the need to establish a registry of DG heterozygotes and to perform robust data analysis to determine if they are at risk, what enzyme level defines that risk, and what dietary intervention, if any, is needed.

Genetic testing for Gaucher Disease: simultaneous detection of common point mutations and pseudogene-derived complex alleles by reverse-hybridization. *G. Kriegshaeuser¹, D. Halsall², A. Moritz¹, F. Kury¹, C. Oberkanins¹.* 1) ViennaLab, A-1110 Vienna, Austria; 2) Dept. Clinical Biochemistry, Addenbrooks Hospital, Cambridge, UK.

Gaucher Disease (GD), the most frequent lysosomal storage disorder, is an autosomal recessive disease characterized by glucocerebrosidase (GBA) deficiency due to mutations in the GBA gene. Accumulation of glucocerebroside, mainly within cells of the monocyte-macrophage lineage, eventually leads to splenomegaly, hepatomegaly, thrombocytopenia, bone marrow suppression, or bone lesions. The disease is panethnic and has been divided into three major types on the basis of the absence (type 1) or the presence and severity of neurologic manifestations (types 2 and 3). The most common variant is type 1 GD, which is particularly prevalent among Ashkenazi Jews (disease frequency 1:850, carrier rate 1:15), and can be efficiently treated by enzyme replacement therapy. We have developed a reverse-hybridization assay (Gaucher Disease StripAssay) for the simultaneous detection of eight common point mutations (84GG, IVS2(+1)A, 1226G, 1297T, 1342C, 1448C, 1504T and 1604A) and two multiply mutated alleles (RecNciI, RecTL) derived from rearrangements between the structural gene and the GBA pseudogene. The test is based on two multiplex DNA amplification reactions and ready-to-use test strips presenting a parallel array of oligonucleotide probes for each wild-type and mutated allele. The entire genotyping procedure from blood sampling to final result requires less than 6 hours, and hybridization and detection may be fully automated on existing equipment (e.g. TECAN profiBlot). We have used this assay to screen a cohort of 91 English GD patients previously genotyped for five mutations by RFLP. While results were consistent for these in all samples, additional GD alleles were detected using the new StripAssay. (oberkanins@viennalab.co.at).

Program Nr: 1244 from 2002 ASHG Annual Meeting

Niemann-Pick Disease Type A and Type B Mutation Detection. *F. Hantash, S. Huang, N. Huynh, W. Sun, C. Strom.*
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Niemann-Pick Disease (NPD) is an autosomal recessive lysosomal storage disorder with significant morbidity and mortality. Mutations in the acid sphingomyelinase (ASM) gene lead to NPD type A and type B. Three mutations (R496L, L302P and fsP330) account for > 95% of mutations in Ashkenazi Jews. A deletion of 3 bp in the codon R608 of the ASM gene leads to NPD type B in homozygotes or in compound heterozygotes with another NPD mutation. We have designed an assay to detect these four mutations using single nucleotide primer extension. After duplex PCR amplification and purification of products, extension primers are added and primer extension is performed using fluorescently-labeled dideoxynucleotides. Purified extension products are separated on ABI 3100 Genetic Analyzer. The use of Biomeck 2000 and ABI 3100 Genetics Analyzer allowed for automated liquid handling and signal detection, and facilitating high throughput performance. Data from ABI 3100 are then analyzed with the aid of GeneScan and Genotyper software and the analyzed results automatically transferred to our patient database. Samples from cultured cell lines from Corriel Cell Repositories with known mutations for type A and type B were used to validate the methodology and 100% concordance was obtained. Furthermore, we exchanged specimens with another reference laboratory and there was 100% concordance. Thus, an automated approach for NPD mutation detection has been developed and validated for clinical patient sample analysis.

Relative incidence of congenital malformations at birth among different religious communities in Israel. . J.

Zlotogora¹, Z. Halai¹, N. Rotem², M. Georgi², I. Berlovitz¹, A. Leventhal¹, Y. Amitai¹. 1) Ministry of Health; 2) Central Bureau of Statistics, Israel.

From 1991 to 2000 there were 1,203,763 live born infants in Israel, including 832,662 Jews, 304,618 Muslims, 26,090 Druzes and 28,915 Christians. The rates of major malformations were in a similar range among Jews or Christians but much higher among Muslims or Druze. The differences may be explained by differences that exist between the communities in particular the rate of consanguinity and of therapeutic abortions. The Muslim and the Druze in Israel are the communities with the highest consanguinity rates (some 50% of the marriages are consanguineous) and the lowest rates of termination of pregnancy even when major malformations are diagnosed. The most significant differences were observed for open neural tube defects that for instance were 4 to 5 times more frequent at birth among either Muslim or Druze than among Jews or Christians. Significant differences were also observed for malformations usually not diagnosed during pregnancy such as congenital heart defects, cleft palate or choanal atresia. For very few malformations such as esophageal fistula and or atresia or small intestine atresia the differences between the communities were not significant.

Analysis of the differences in the rate of malformations at birth in different religious communities may help to delineate etiologic factors and serve as a basis for Public Health policies.

Prevalence survey of the Fragile X E syndrome referred for Fragile X syndrome testing in boys with mental retardation. *S. Chen^{1, 2}, J.M. Schoof¹, C.J. Lemoine¹, C.L. Gordon¹, C.R. Scott^{1, 2}.* 1) Molecular Diagnostic Lab, Children's Hosp/Reg Med Ctr, Seattle, WA; 2) Department of Pediatrics, University of Washington, Seattle, WA.

Fragile X E syndrome (FRAX E) is associated with mild to borderline (I.Q.= 50-80) mental retardation with no specific and consistent dysmorphology. For last 8 years, we have surveyed mentally retarded boys for FRAX E. The blood samples were sent to the lab for FRAX syndrome testing from clinics throughout the northwestern United States. After PCR and electrophoresis, then a chemilluminescence detecting method was used to identify both the number of the CCG repeats in FRAX E gene and the CGG repeats in FRAX A gene. Out of 1812 samples tested for FRAX E, we found 2 patients with >200 CCG repeats. Twenty-seven patients out of 1805 samples tested were identified with FRAX A. If we accept that the prevalence of FRAX A is about 1/4,000, then the prevalence of FRAX E could be about 1/60,000 in the population. Because the mild nature of FRAX E, the real prevalence might be higher than this estimation. The number of positive FRAX E patients in this sample is too small to make an accurate value. We will continue to compile the data in years to come.

During further studies on a three-generation pedigree in one of our FRAX E families, we found that, in contrast to FRAX A, the transmitting FRAX E male had resulted in a contraction of the CCG repeat in his daughter. The repeat then expanded in his grandson who had mild mental retardation. Current technology allows us to test expansion of FRAX A and E simultaneously, and a more accurate estimation of prevalence of FRAX E large alleles associated with mental retardation can be expected in the future.

A140V is a recurrent mutation in the MeCP2 gene that may be systematically screened in patients with idiopathic mental retardation. *B. Winnepenninckx¹, V. Errijgers¹, F. Hayez-Delatte², E. Reyniers¹, R.F. Kooy¹.* 1) Dept. of Medical Genetics, University of Antwerp, Antwerp, Belgium; 2) Hôpital Erasme, ULB, Brussels, Belgium.

Mutations in the methyl-CpG-binding protein 2 (MeCP2) cause Rett syndrome, a severe neurodevelopmental disorder occurring predominantly in females. Male patients with Rett syndrome are extremely rare as the Rett causing mutations in the MeCP2 gene are lethal in hemizygous males. In addition, different variations in the same gene were reported to cause mental retardation, both in sporadic non-syndromic males as in syndromic families with disease manifestation in carrier females. However, the majority of the reported MeCP2 mutations in mental retarded patients consist of amino acid substitutions and, especially in isolated cases, discrimination between a disease causing mutation and a rare polymorphism is not obvious and the significance of each individual variation should be verified. We mapped a new non-syndromic X-linked family (MRX79) to Xq27.3-28 and found an A140V mutation in the MePC2 gene in all patients with the disease haplotype. In addition to data published by others, this suggests that A140V is a recurrent mutation (and not a polymorphism) in patients with mental retardation. As a consequence, implementation of the A140V mutation in routine screening programs of the intellectually disabled should be considered.

Development of *Alu* PCR and *Alu* Real-Time PCR Methods for Quantitation of Human DNA in Forensic Samples. *J.A. Nicklas, E. Buel.* VT Forensic Laboratory, Department of Public Safety, Waterbury, VT.

There are a number of steps required to obtain a DNA profile from a crime scene sample. One critical step in this process is the determination of the amount of human DNA contained in the sample. The forensic community relies predominately upon a technique (slot blot) to quantitate human DNA that is imprecise, time consuming and very labor intensive. We have developed new, faster and more quantitative techniques based on PCR of the primate-specific *Alu* sequence that is found in over 800,000 copies in the human genome. Primers were used to amplify a 124bp fragment of *Alu* sequence in a reaction containing SYBR Green I stain. Quantitation was performed in a fluorescent plate reader (Bio-Tek FL_x800). This plate reader assay consisted of an initial zero time SYBR Green I fluorescence reading, 14 cycles of PCR and then a final fluorescence reading. Subtracting the initial time reading from the final reading removed the fluorescent contribution from the input DNA. Fluorescence background was decreased by using QSY7 labeled primers (method patented by Molecular Probes, Inc.). The QSY7 moiety quenches the background SYBR Green I fluorescence of the primers and of primer-dimers. We have explored a number of variables for this assay including: number of PCR cycles, multiple readings, annealing temperature, annealing time, extension time, specificity (test of animal and bacterial DNAs), Mg⁺⁺ concentration and SYBR Green I concentration. This assay has a dynamic range of 10ng to 10pg in a 25ul assay and is specific for human (or other primate) DNA. We have also performed experiments using *Alu* PCR in a real-time format also using SYBR Green I stain. These results indicate that this assay is also specific for human DNA and has a range of 16ng to 3.9pg in a 10ul assay. Our results indicate that use of *Alu* PCR can be a quantitative, fast and inexpensive way to quantitate human DNA for forensic or other studies. [Supported in part under award 2000-IJ-CX-K012 from the Office of Justice Programs, Natl. Institute of Justice, Dept. of Justice. This abstract does not necessarily represent the official position of the U.S. Department of Justice.].

The use of a 5 fluorogenic real time PCR assay for the specific quantitation of amounts of mtDNA in DNA

extracts. *J.G. Lorenz¹, E.K. Lin^{1,2}, W.R. Beggs¹, J.C. Beck¹, P.K. Bender¹*. 1) Coriell Inst Medical Research, Camden, NJ; 2) Brandeis University, Waltham, MA.

The use of mitochondrial DNA (mtDNA) in clinical, forensic and population genetic studies has become widespread within the last decade. Generally total DNA is extracted from the relevant biomaterial and total quantity of DNA (nuclear and mtDNA) is determined using any of several methods. When working with high quality biomaterials total DNA yields are generally sufficient and it may not be necessary to quantify the amount of mtDNA specifically. However, there are cases when it is desirable to know how much, if any, mtDNA is present in a sample either before or after a procedure. For example, when working with DNA extractions from bone, tissue or other portions of human remains from archaeological sites it is valuable to know whether or not any mtDNA is present in a given sample extract and thus whether it is worth investing the time and effort to proceed with the analysis. It is also of interest to know the relative contribution of the mtDNA component to the total DNA concentration from extracts from various sources such as mouthwash and whole genome amplified (WGA) materials. We have developed a 5 fluorogenic real time PCR assay using primers and a fluorescently labeled probe that targets a 70 bp segment of the mitochondrial cytochrome B gene. Using a standard derived from a plasmid containing a portion of the cytochrome B gene of known quantity it is possible to quantify the amount of mtDNA present in DNA extracted from a variety of sources including urine, plasma, buccal swabs, mouthwash, hair, blood spots, bone, teeth and various tissues. Using this technique it is possible to detect approximately 0.4 picograms of mtDNA in heterogeneous extracts containing various quantities of mitochondrial, nuclear and bacterial DNA. Although the current primer/probe combination is specific to human mtDNA it could easily be modified to detect a wider range of species.

Proficiency testing scheme results point to the need for a better validation of newly introduced methodologies in routine genetic testing. *E. Dequeker¹, E. Girodon², M. Schwarz³, M. Stuhmann⁴, J.J. Cassiman¹.* 1) Department of Human Genetics - University of Leuven, Belgium; 2) Service de Biochimie et Genetique Moleculaire, Hopital Henri Mondor, Creteil, France; 3) Paediatric Genetics Unit, N.W. Regional Molecular Genetics Laboratory, Manchester, United Kingdom; 4) Human Genetics, Hannover Medical School, Hannover, Germany.

DNA-based testing for genetic diseases has developed from nothing into a general part of laboratory medicine. Testing for genetic disorders is usually carried out in molecular genetic diagnostic laboratories. However, as commercial kits become available, molecular genetic testing for more common inherited disorders such as cystic fibrosis is increasingly being carried out in general pathology laboratories and commercial centers. External quality assessment (EQA) schemes (or proficiency testing) for cystic fibrosis have been organized over the past six years with 135 to 206 participating diagnostic laboratories. A significant improvement of the quality of genotyping results was obtained during subsequent QA schemes, coming from 65% of laboratories without errors in 1996 up to a more or less constant level of approximately 90% since 1999. During these years new commercial kits and methodology became available and were being applied in many diagnostic laboratories. However, the use of a commercial kit alone does not ensure high accuracy of mutation analysis: results of the EQA schemes demonstrated that laboratories which implement a new commercial kit or methodology for mutation analysis of cystic fibrosis (e.g. OLA, INNO-Lipa, Elucigene, DHPLC) made more mistakes during the implementation period. Nevertheless, these methods were already being used for mutation detection in their routine laboratory. External quality assessment schemes and the use of reference materials or certified test samples are possible routes to help the laboratories to improve their quality. Laboratories should be aware of the importance to implement quality assurance systems that include validation of the used procedures.

Mutation analysis of the early growth response 2 gene (*EGR2*) in a cohort of 5,000 patients referred with neuropathy. *J.A. Curran*¹, *U. Ananth*¹, *P. Smith*¹, *G.M. Saiji*², *J.R. Lupski*², *W.K. Seltzer*¹. 1) Research and Development, Athena Diagnostics Inc., Worcester, MA; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030.

Charcot-Marie-Tooth disease Type 1 (CMT1) is a genetically heterogeneous neuropathy that results from an underlying defect in peripheral nerve myelination. Genes directly involved in myelination and shown to be pathologically associated with CMT1 include *PMP22*, *Cx32*, *MPZ* and *EGR2*. The early growth response 2 (*EGR2*) gene, encodes a zinc-finger DNA-binding transcription factor that is essential for the regulation of gene expression during myelination. Among patient specimens submitted for CMT1 mutation analysis, Athena Diagnostics, Inc. has analyzed over 5,000 DNA specimens for sequence variations within the *EGR2* gene using automated DNA sequencing. The entire coding region of the *EGR2* gene, including the highly conserved exon-intron splice junctions, was amplified as two PCR products and bi-directionally sequenced in four fragments. We have identified 38 new sequence variants and 5 previously reported variants. Of the 38 newly described variants, 26 were missense mutations, 1 was a 3bp in-frame insertion, and 11 were synonymous codon mutations. The known A362C common polymorphism was observed in 4.23% of patients and a second newly described variant A209G was observed in 0.54% of patients. The remaining variants were found to recur less than 5 times in the population tested. Sequence variants were found to be clustered in three regions of the *EGR2* gene; just prior to the Inhibitory Domain, within the zinc finger domain and just after the zinc finger domain. A list of the *EGR2* variants detected will be presented including the first variant detected in exon 1 of *EGR2*, and several new variants which may affect the zinc finger domains. Predicted functional consequences of selected mutant alleles will be presented.

Comparison of Direct Sequencing to Other Mutation Detection Methods for the TSC1 and TSC2 Genes. *K.-S. Au, A. Tucker Williams, M.W. Bishop, H. Northrup.* Dept Pediatrics, Univ Texas Medical Sch at Houston, TX.

Tuberous sclerosis complex (TSC) is a dominantly inherited disease of benign tumors occurring approximately 1 in 10,000 in the population. The diagnosis in clinically apparent affected individuals can easily be determined, but there is a demand/need for molecular testing in individuals with subtle findings as well as family members of affected individuals. Two genes, TSC1 and TSC2, have been identified as causative; however, development of a satisfactory mutation screening strategy for diagnostics has proven problematic. There are 62 coding exons with small mutations scattered throughout accounting for the majority (85%) of identifiable mutations resulting in the need to use detection methods that are labor-intensive. Further complicating testing is a significant percentage (8-15%) of large gene deletions/rearrangements in the TSC2 gene and a percentage of patients with no detectable mutation (7-13%). We tested direct sequencing as a potential method with a protocol beginning September 2000. We will compare our sample set for sensitivity and cost to other methods (SSCA, HA, and DHPLC) reported in the literature. We expect a total of 250 samples from definitively diagnosed individuals to be completed by September 2002. To date, 126 samples have been sequenced. Of the 126, 102 (89 with a Definite diagnosis) newly ascertained samples are completed, 11 previously screened as negative by SSCA are completed and 13 samples had variants (mutation v. harmless polymorphism) identified requiring further testing. Sensitivity of other methods have been reported as 61% (SSCA), 58% (HA) and 68% (DHPLC). Among our completed newly ascertained cases with a Definite diagnosis, mutations were detected in 69/89 (77.5%), the highest reported detection rate utilizing a single methodology. We identified mutations in 4/11 samples that were screened negative by SSCA. Our projected cost analysis based on testing 150 samples/year is approximately \$659/sample compared to other methods at \$373 (SSCA), \$414 (HA) and \$566 (DHPLC). Final results will be presented for the entire sample set assessing sensitivity and cost.

Mutational screening of Italian Wolfram Syndrome patients by DHPLC. A. Colosimo^{1,2}, V. Guida^{1, 3}, L. Rigoli⁴, C. Di Bella⁴, A. De Luca¹, G. Palka², D.C. Salpietro⁴, B. Dallapiccola^{1,3}. 1) CSS Mendel Institute, Rome, and CSS Hospital, San Giovanni Rotondo, Italy; 2) Department of Biomedical Sciences, University of Chieti, Italy; 3) Department of Experimental Medicine and Pathology, University La Sapienza, Rome, Italy; 4) Department of Paediatrics, University of Messina, Italy.

Background: Wolfram Syndrome (WS) is an autosomal recessive neurodegenerative disorder also referred to as DIDMOAD, since characterized by diabetes insipidus, diabetes mellitus, optic atrophy and deafness. The causative gene for WS (WFS1), mapped to chromosome 4p16.3, consists of eight exons, spanning 33.44 Kb of genomic DNA. A wide range of mutations and polymorphisms have been described in WFS1, so far. In this study we report on the mutational analysis of the WFS1 gene in a total of 19 Italian WS patients and 25 relatives, using a Denaturing High Performance Liquid Chromatography (DHPLC)-based protocol. Methods: The entire WFS1 coding region was amplified in 14 fragments of 220-400 bp and analyzed by DHPLC. We established the optimal DHPLC parameters of each coding exon using the WAVE Maker Software version 4.1.40. Each anomalous elution peak was then subjected to direct sequencing. Results: Our protocol enabled us to identify the causative mutations in all WS patients and the inherited WFS1 alleles within each nuclear family analyzed. Several novel mutations as well as neutral changes and uncoding polymorphisms were identified, mostly of them being localized in WFS1 exon 8. Neither false positive nor false negative DHPLC results were observed. Conclusions: The DHPLC-based protocol we have developed can be used for an efficient, cost-effective and reliable mutational analysis of the WFS1 gene. In addition, the present study represents the molecular characterization of the largest cohort of Italian WS carriers and patients studied so far and expands the spectrum of WFS1 allelic variants worldwide.

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Development of an Automated PCR-RFLP genotyping assay for the β 3-Adrenergic Receptor Gene. *U.R. Desai, C. Song, S.M. Sell.* Dept. of Nutrition Science, Univ. of Alabama at Birmingham, Birmingham, AL.

The β 3-adrenergic receptor is expressed in visceral adipose tissue and is assumed to play a role in the regulation of lipolysis and thermogenesis. A polymorphism in the β 3-adrenergic receptor gene at codon 64 is characterized by the replacement of tryptophan by arginine (Trp64Arg polymorphism) and is associated with the development of obesity and non-insulin dependent diabetes mellitus. Consequently, many gel-based assays have been developed for the detection of this polymorphism. Presented here is an alternative to the conventional PCR-RFLP assays, which use polyacrylamide or agarose gel electrophoresis. Here we have developed a fast and accurate PCR-RFLP based method, using automated capillary electrophoresis, for detecting the Trp64Arg polymorphism of the β 3-adrenergic receptor. DNA was amplified with specific PCR primers that flanked the Trp64Arg polymorphism of which the forward primer was labeled with a fluorophore, TET. The resulting product was then incubated with the restriction endonuclease, Bstn1 which distinguishes between the normal and mutant sequences. DNA fragment analysis was then carried out using an automated, capillary electrophoresis system with laser detection (ABIPrism310). We hope to apply this new assay to larger population studies, which are needed to corroborate and extend the evidence for disease association. This work was supported by NIH grant #P30DK056336.

Program Nr: 1255 from 2002 ASHG Annual Meeting

Development of a High Throughput Comprehensive Sequencing Assay for CFTR Gene Mutation Detection. *S. Huang, C. Chen, M. Peng, B. Anderson, M. Zoleikhaian, S. Olson, W. Sun, CM. Strom.* Dept Molecular Genetics, Quest Diagnostics Inc., San Juan Capist, CA.

Cystic Fibrosis (CF) is the most common lethal autosomal recessive disease among Caucasians and also affects smaller proportions of all other ethnic groups. >1000 mutations have been reported throughout the 27 exons and 26 introns in CFTR. The ACMG panel for CF carrier screening will detect both CF mutations only in <65% of CF patients. For these patients, accurate and comprehensive detection of mutations in CF is important for genetic counseling of relatives, prenatal diagnosis and genotype-phenotype correlation. We developed a full sequence analysis assay for all CFTR exons, splice sites and the promoter region using automated capillary sequencing. Results: The assay was validated using 18 patients with positive ACMG screening results. After blinding the samples, we confirmed all the screening results using our sequencing assay. For 5 affected patients with at least one CF allele not identified by carrier screening, our sequencing assay identified all the CF chromosomes. The genotypes were: F508/R1066C, homozygous 3154delG, F508/P205S, R553G/L1335P and L138ins/3272-26A->G. In addition we were able to resolve 5 ambiguous results of carrier testing using sequence analysis. Conclusion: Complete sequencing of CFTR will be an important adjunct to current methodologies for CF patients and their families.

A unified approach to diagnostic testing for single gene disorders. *L.S. Horrocks¹, L. Steele^{1,2}, N.L. Bulgin¹, T.L. Stockley^{1,3}, P.N. Ray^{1,2}.* 1) Molecular Genetics Laboratory, Department of Paediatric Laboratory Medicine, The Hospital for Sick Children; 2) Department of Molecular and Medical Genetics; 3) Department of Laboratory Medicine and Pathobiology, University of Toronto, Ontario, Canada.

Over 13,500 genetic disorders are listed in Online Mendelian Inheritance in Man, half of which are associated with single gene disorders. As a hospital-based Molecular Diagnostic Laboratory, establishing and executing a unique test for each of these disorders is logistically impossible and prohibitively expensive. We have developed a unified approach for identifying private mutations in rare diseases (i.e. Fabry, Hunter and Retinoblastoma) and have applied it to the diagnosis of relatively more common conditions (i.e. Duchenne/Becker MD). This strategy employs a modular approach using the following techniques 1) Quantitative Multiplex-PCR analysis (QMP) to look for gene deletions or insertions 2) Denaturing High Pressure Liquid Chromatography (DHPLC) to screen for small nucleotide changes followed by 3) Direct Sequencing (DS). These techniques are easily adaptable for use in any diagnostic laboratory setting and can be applied to the diagnosis of many single gene conditions. Depending on the frequency and types of mutations that cause disease, the modules are interchangeable in order to provide the most cost-effective strategy of mutation detection. Once the familial mutations have been identified, direct mutation detection in other family members is greatly simplified.

A Diagnostic Strategy for Molecular Genetic Testing of Hunter disease. *G. Koulchitski¹, L. Steele^{1,2}, Y. Yang¹, Q. Yi¹, T.L. Stockley^{1,3}, P.N. Ray^{1,2}.* 1) Department of Paediatric Laboratory Medicine, The Hospital for Sick Children; 2) Department of Molecular and Medical Genetics;; 3) Department of Laboratory Medicine and Pathobiology, University of Toronto, Ontario, Canada.

Hunter disease (MPS2) is a rare X-linked lysosomal storage disease with an incidence ~1 in 100,000 male births due to a deficiency of the enzyme iduronate-2-sulphase. The phenotype can vary from mild to severe depending on the level of enzyme deficiency and type of mutation in *IDS* gene, located at Xq28. Approximately 2/3 of mother of affected males are carriers of Hunter disease. A pseudogene, *IDS-2*, also exists telomeric to the *IDS* gene and shares a high degree of homology to the *IDS* gene. Many disease causing mutations have been reported in *IDS* gene including whole or partial gene deletions (10%), gene rearrangements due to recombination between the *IDS* gene and the *IDS-2* pseudogene (10%) and nucleotide substitutions (missense/nonsense/splice site) or small insertion/deletions (ins/dels) (80%). The objective of our study was to develop a cost-effective diagnostic strategy for the diagnosis of patients with Hunter disease using 1) Quantitative Multiplex-PCR (QMP) to look for gene ins/dels 2) Southern blot (SB) analysis to detect gene rearrangements and 3) Direct sequencing (DS) for point mutations & small ins/del. DNA was tested from 10 probands with Hunter disease, all of which were found to have mutations in the *IDS* gene. Seven had small mutations in the *IDS* gene picked up by direct sequencing which include missense (2), splice site (3) and small ins/dels (2). Two of the patients had partial gene deletions while a gene rearrangement was detected in one patient. All 4 mothers for which DNA was available were carriers of the family mutation. Based on these results, a sequential diagnostic strategy was developed which provides accurate diagnosis and prenatal diagnosis in families with Hunter disease.

Multicenter validation of a new reverse hybridization assay for CFTR mutation analysis. *L. Messiaen¹, M. Stuhmann², P.F. Pignatti³, G. Matthijs⁴, J. Poncin⁵, P. Cochaux⁶, P. Hendrix⁷, G. Verpooten⁷, A. Devos⁷, A. Verhelst⁷, C. Van Loon⁷.* 1) Dpt. of Medical Genetics, Ghent University Hospital, Ghent, Belgium; 2) Medizinische Hochschule Hannover, Hannover, Germany; 3) University of Verona, Institute of Biology and Genetics, Verona, Italy; 4) Center for Human Genetics, University of Leuven, Leuven, Belgium; 5) CHU de Liège Génétique, Domaine Universitaire Sart Tilman, Liège, Belgium; 6) ULB Hôpital Erasme, Laboratoire de Génétique Moléculaire, Brussels, Belgium; 7) Innogenetics NV, Gent, Belgium.

The performance of the INNO-LiPA CFTR12 and INNO-LiPA CFTR17+Tn for the detection and identification of 29 specified cystic fibrosis transmembrane conductance regulator (CFTR) mutations and their wild-type sequences, as well as the identification of a congenital bilateral absence of the vas deferens (CBAVD)-related polymorphism, were validated in a European multicenter study. Each center selected approximately 40 samples with a known mutation profile and 10 wild type control samples to be tested with the INNO-LiPA CFTR assays. A total of 364 CF and control samples were amplified, 360 of which were analyzed on the INNO-LiPA strips. For these samples, a 99.2% concordance with conventional genotyping methods was found. This was further improved to 100% after optimization of 4 probe concentrations on the LiPA strips. The specificity of the INNO-LiPA CFTR was further investigated at Innogenetics by testing possible cross-reactivity of benign polymorphisms or rare CF mutations located near or at the location of the mutations for which probes are present on the INNO-LiPA strips. No cross-reactivity was found. In conclusion, this study shows that the INNO-LiPA CFTR12 and INNO-LiPA CFTR17+Tn detect and reliably identify an important number of CF-related mutations, as well as the CBAVD-related Tn polymorphism in the CFTR gene region. For the U.S. market, a new strip was designed containing the probes already present on the CFTR12 strip, and to which 4 additional mutations recommended by the American College of Medical Genetics were added.

Results of reference testing of EBV transformed cell lines for performance evaluation and quality assurance in molecular genetic testing. *L.O. Williams¹, A.K. Stankovic¹, S. Bernacki², J.C. Beck³, K. Snow², V. Pratt², K. Monaghan⁴, K. Matteson⁵, F. Schaefer⁶, M. Friez⁷, A. Shrimpton⁸, D. Farkas⁹, T. Stenzel².* 1) Centers for Disease Control and Prevention, Atlanta, GA; 2) Duke Univ Med Ctr, Durham, NC; 3) Coriell Institute for Med Research, Camden, NJ; 4) Henry Ford Hosp; 5) Univ TN Med Ctr; 6) Chapman Inst Med Genetics; 7) Greenwood Genetic Ctr; 8) SUNY Upstate Med Univ; 9) Baylor Col Med.

The role of human molecular genetic testing (hMGT) expands as the number of mutations associated with disease increases. The need for stable sources of positive controls for performance evaluation and quality assurance (PE/QA) of hMGT persists. The CDC and Duke Med. Ctr., in collaboration with Coriell Inst., are conducting a project to establish stable EBV-transformed cell lines from residual blood samples containing targeted mutations associated with diseases of public health importance. The first set of ten cell lines were established from residual samples provided by consultants associated with the project or from pre-existing Coriell samples. After stable transformation of cell lines, frozen cell pellets were sent to outside laboratories for reference testing. Each sample was tested by five or more laboratories, most of which perform clinical testing for the targeted mutation. Targeted mutations were point mutations or small deletions. Eight laboratories participated. Each mutation was reference-tested by two or more technologies typically used in clinical laboratories for detecting that mutation. Seven different nucleic acid purification techniques and fifteen different detection techniques were used. The techniques used were dependent upon the targeted mutation. The presence of targeted mutations was confirmed in all cell lines regardless of nucleic acid purification technique or detection technique. In initial stability testing of other cell lines containing triplet repeat expansions, the number of repeats was constant through five culture passages and twenty population doublings. The transformation and verification process used in establishing these cell lines represents a successful approach for producing positive samples for PE/QA uses.

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Molecular Diagnostic Testing for Autosomal Dominant Hereditary Spastic Paraplegia: Identification of Novel mutations in the *SPG4* gene. J. Wang, A.N. Hennigan, A. Morini, U. Ananth, W.K. Seltzer. Athena Diagnostics, Inc., Worcester, MA 01605.

Hereditary Spastic Paraplegia (HSP) type 4 is the most common form of autosomal dominant HSP, a neurodegenerative disease characterized by progressive spasticity of the lower limbs and hyperreflexia. Studies have shown that mutations in the *SPG4* gene are responsible for approximately 40% of all diagnosed HSP cases. The *SPG4* gene maps to chromosome 2p21-p22 and encodes Spastin, a putative nuclear protein of the AAA (ATPases associated with diverse cellular activities) family. Molecular diagnostic testing for *SPG4* mutations is performed by DHPLC (denaturing high performance liquid chromatography) and automated DNA sequencing. The entire coding region and highly conserved splice sites of the *SPG4* gene were amplified as 17 gene segments (exons 1-17). Six of the 17 segments were sequenced directly in forward and reverse strands. The remaining 11 segments were screened by DHPLC and positives from the screen were further analyzed by bi-directional sequencing. Analysis of 223 DNA specimens submitted to Athena Diagnostics, Inc. for *SPG4* gene mutation identification yielded 30 sequence variants predicted to alter the primary structure of Spastin. Twenty three of these were novel mutations and 7 were mutations previously reported in the literature. The novel variants identified consisted of 6 nonsense, 12 missense, 4 deletions (one splice site) and 1 insertion. Interestingly, of these 23 new variants, 18 (82%) were located in the AAA cassette region, a previously reported protein functional domain. This study provides a spectrum of novel *SPG4* mutations, and implicates the AAA cassette region as critical to Spastin function.

Familial dysautonomia mutation frequency: clinical testing of greater than 2700 specimens confirms high frequency in Ashkenazi Jews. . E.A. Sugarman, B.A. Allitto. Genzyme Genetics, Framingham, MA.

Familial dysautonomia (FD) is an autosomal recessive neuropathy occurring almost exclusively among Ashkenazi Jews. Autonomic and sensory nervous system dysfunction lead to variable symptoms including decreased sensitivity to pain and temperature, labile blood pressure, vomiting, gastrointestinal problems, lack of overflow tears, hypotonia, and scoliosis. FD is progressive and fatal; 50% of patients survive to age 30. The responsible gene, IKBKAP, and two mutations accounting for >99.5% of alleles were recently identified.

An FD carrier frequency of 1/30 among Ashkenazi Jews, similar to that of Tay-Sachs, has been estimated from disease incidence. We report our experience in offering clinical testing for the 2 reported mutations: 2507+6T>C and R696P. From October 2001 to May 2002, 2,743 specimens were referred for FD mutation analysis. Of these, 2728 were for carrier testing: 2702 with no family history; 26 with a family history. Among 2148 individuals referred for carrier screening and self identified as Ashkenazi Jewish, 78 were carriers of 2507+6T>C and 1 was a carrier of R696P. The carrier frequency based on our testing was 1/27 - similar to the 1/30 predicted by disease frequency. Of 554 carrier screening individuals reporting either partial or no Ashkenazi Jewish ancestry, 4 were 2507+6T>C carriers (1/138). Prenatal diagnosis was performed for 7 fetuses: 5 fetuses (3 carriers; 2 noncarriers) from 3 families with a previous affected child; 2 fetuses (both negative) with one carrier parent. Of eight referrals for a suspected diagnosis (3 Ashkenazi, 5 other ethnicities), one Ashkenazi patient was homozygous for 2507+6T>C and the others were negative.

Our clinical testing experience confirms the high carrier frequency of FD in the Ashkenazi Jewish population. Current ACOG standard of care is to offer carrier testing for CF, Tay-Sachs and Canavan disease to Ashkenazi Jewish individuals. Given the significant morbidity of FD and a detection rate comparable to these diseases, the addition of FD to carrier testing recommendations may be warranted.

EPM1 methylation-analysis improves PCR testing of myoclonus epilepsy - type Unverricht Lundborg. A.S.

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Progressive myoclonus epilepsy of the Unverricht-Lundborg type (EPM1; MIM 254800) is an autosomal recessive disorder caused by loss of cystatin B gene function. In the vast majority of cases, the loss of gene activity is caused by EPM1 dodecamer repeat expansions affecting both alleles within the 5-gene region, disabling proper mRNA transcription. Diagnostic procedures therefore rely on the determination of the extent of the repeat expansion. Up to now, this has been achieved with Southern blot analyses and PCR; due to the high CG content of the EPM1 region conventional PCR amplification of affected alleles fails and enhanced technical effort is necessary to overcome amplification failure. To resolve some of these problems, we have developed a methylation-sensitive PCR-test on sodium bisulfite treated DNA. We have designed primer sets that cover the methylated and non-methylated promoter, and the deaminated EPM1-repeat. With promoter primer-sets we are able to show that the normally unmethylated promoter region does not change along with repeat expansion. Thus unlikely fragileX syndrome, methylation testing does not enable indirect diagnostic testing for the EMP1 repeat expansion and the syndrome. In addition to determination of the methylation status in affected patients, we used bisulfite treated DNA for amplification of the EMP1 repeat expansion and could establish a novel PCR test for direct testing of EMP1 repeat expansion. Thus we were able to amplify the affected alleles in a series of full mutation patients and also within the heterozygous full mutation carriers, who are prone to misdiagnosis due to PCR-product competition of the normal allele. Our MS-PCR approach, thus provides proper PCR testing and would replace laborious Southern-blot analyses.

Modeling of periodic screening of smokers with increased genetic susceptibility to lung cancer: Impact on population-based mortality. *M. Kimmel*¹, *O. Gorlova*², *C. Amos*², *M. Spitz*², *C. Henschke*³. 1) Dept Statistics, Rice Univ, Houston, TX; 2) Department of Epidemiology, University of Texas MD Anderson Cancer Center, Houston, TX; 3) New York Presbyterian Hospital-Weill Medical College, Cornell University, New York, NY.

Previous studies indicate that genetic factors play a role in lung cancer susceptibility (reviewed in Amos et al., *Chemoprevention of Cancer*, 1999:3-12). We consider a population of individuals at high risk for lung cancer (smokers), additionally stratified by a genetic susceptibility variate. We use a stochastic model of disease progression and detection to estimate the mortality reduction associated with early detection of lung cancer followed by appropriate treatment. The estimates of model parameters have been obtained based on data from previous screening studies (Gorlova et al, *Cancer* 2001, 92: 1531-1540). Two theoretical possibilities are considered: (1) The highly genetically susceptible (HGS) smokers do not differ, with respect to the time-course of disease, from other smokers; (2) The HGS smokers have an earlier onset and/or faster progressing disease. Preferential screening of the HGS smokers is advisable based on cost-effectiveness considerations. Under scenario 2, the screening has to start earlier to ensure a meaningful reduction of mortality.

We evaluated our modeling approach using data from an ongoing case-control lung cancer study conducted in the Epidemiology Department at the MD Anderson Cancer Center. Smokers with upper quartile outcomes of both bleomycin and BPDE mutagen sensitivity assays represent a higher fraction of cases than controls. From these data, it can be estimated that the lifetime susceptibility to lung cancer of these HGS smokers equals approximately 0.5, as opposed to approximately 0.09 for the other smokers. Based on our calculations, although the HGS smokers constitute only around 10% of all smokers, they account for approximately 40% of lung cancers in the population.

Chromosomes 2 & 7 in AutD: Clinical comparisons of phenotypic subtypes. *S. Donnelly¹, C. Wolpert¹, L. Elston¹, S. Ravan², K. Decena², R. Abramson², H. Wright², M. Cuccaro¹, M.A. Pericak-Vance¹.* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) WS Hall Psychiatric Institute, Univ of South Carolina, Columbia, SC.

Autism (AutD) is a complex neurodevelopmental disorder characterized by social-communicative impairments and repetitive behaviors. Multiple genes may have etiologic relevance to AutD with at least 10 different genes suspected. Chromosomes 7 and 2 have demonstrated the strongest linkage to date when phrase speech delay (PSD) has been used to subset families. We used ordered subset analysis (OSA) to identify subsets of families for both Chr2 and Chr7. OSA is a statistical procedure in which a clinical covariate is used to identify a homogeneous subset of families that contribute to linkage in a chromosomal location. Using PSD (phrase speech onset >36 months) as a covariate, OSA identified 29 families for Chr7 and Chr2. All Chr7 families were in the Chr2 subset (n=17); a subset of Chr2 families were only linked to that chromosome (n=12). We compared the Chr2 and Chr7/2 subsets on the Autism Diagnostic Interview-Revised (ADI-R), Aberrant Behavior Checklist (ABC), and Vineland Adaptive Behavior Scales (VABS). The groups failed to differ on the ADI-R social algorithm total and repetitive behavior scores as well as the ABC Lethargy factor. However, the two groups differed significantly across each of the VABS indices. The Chr2 group showed consistently higher scores in social (p=. 0004), communication, (p=. 0189), and daily living skills (p=. 0006) domains as well as in overall adaptive functioning (p=. 0004). Further, the Chr2 group also demonstrated less impairment on two ADI-R measures of communication: gestures (p=. 003) and social imitative behaviors (p=. 0015). These findings suggest that the Chr2 and Chr7/2, despite being stratified on PSD, are distinctly and consistently different on several clinical dimensions including additional measures of communication. This shows that the Chr7/2 subset is more impaired than the individuals in the Chr2 subset alone. In addition, the Chr2 individuals may show a different trajectory of development particularly in language and communication.

Confidential pre-employment counseling and genetic marker screening. *L. Godmilow*¹, *D. Duebner*². 1) Genetics, University of Pennsylvania, Philadelphia, PA; 2) Occupational Medicine, Brush Wellman, Inc, Cleveland, OH.

Beryllium, an element mined commercially, is used in electronics components, fiber optics components, nuclear weapons, nuclear reactors and other important products. Brush Wellman, Inc (BWI) is a major producer of high-performance engineered beryllium materials which involves processes that produce fine beryllium particles. Workplace contact with beryllium particles may result in a severe inflammatory lung disease, chronic beryllium disease (CBD). There is evidence to suggest that a particular HLA marker, Glu⁶⁹, might increase the risk of developing CBD.

BWI approached the University of Pennsylvania about designing a pilot program to make confidential genetic counseling and HLA marker testing available to a group of persons considering employment at the BWI plant in Tucson, AZ. Current employees and outside scientific advisors supported the concept of making this testing available. IRB approval was obtained.

Sixty consecutive individuals who had been offered employment were given the opportunity to participate in the program. They were provided with a toll-free telephone number to call the genetic counselor if they wished to initiate genetic counseling and explore further the option of testing. Twelve persons were counseled and nine had their blood samples submitted (15% of the original 60 subjects). Of the 9 samples submitted, two (22%) were positive for the HLA Glu⁶⁹ marker and seven (78%) were negative.

All prospective employees who were counseled said they were going to accept the job offer (and all did) despite a positive result but were interested in knowing if they carried the HLA marker. Employment at the BWI plant is the highest paid in the Tucson area for unskilled workers. Counselees were positively impressed by the company's efforts to minimize exposure to beryllium and seemed to feel their risk to develop CBD was exceedingly small even if they carried the marker. The program is being considered for revision and expansion to two additional company plants.

Media Portrayal of Genetic Discoveries: A Comparison of Newspaper Stories and Peer Reviewed Research Papers. *T.A. Caulfield, T. Bubela.* Health Law Inst, Univ Alberta, Edmonton, AB, Canada.

Many commentators have speculated that the media inappropriately "hypes" the portrayal of genetic research. Likewise, numerous studies have reported that the popular media, including newspapers, are a primary source of genetic information for the public and health care professionals. It is also likely that media coverage of social issues associated with genetic discoveries has an impact on public opinion and an indirect impact on policy development. We will present a comprehensive review of existing international survey data on where the public, clinicians, and policy makers receive their information about genetic discoveries. In addition, we review existing and present original data on the manner in which genetic stories have been presented in the media since 1995. We examine 1330 newspaper articles in 28 newspapers in Canada, the UK, Australia, and the United States, reporting on 153 studies published in the primary scientific literature from 1995-2000. The focus is on the reporting of single gene discoveries. Using a standard questionnaire and project naive coders, we compare the accuracy of the description of the scientific research and the claims and conclusions made in the primary research article with those made in the newspaper article. We compare the level of hype associated with positively viewed discoveries such as disease genes and potential treatments with more contentious news items surrounding reproductive technologies and behaviour genes. We found that gene discovery stories are highly localized and generally have a short longevity in the media. Claims are more likely to be exaggerated in stories on reproductive technologies and behaviour genes than disease genes. Gene discoveries with the greatest longevity in the media are associated with the most social commentary and misinformation. Such ill-informed policy debates amplify many of the fundamental concerns by reifying genetic material and confirming notions of genetic essentialism. This impacts formal policy and regulatory responses to genetic research, many of which seem to be misplaced reactions to the hyperbole instead of thoughtful, sustainable, regulatory strategies.

Obstetricians Initially Appear to Find the ACOG/ACMG Cystic Fibrosis Screening Guidelines Difficult to Implement. *S.L. McAdoo, J.L. Simpson, A. Burke, M. Totorica.* Obstetrics & Gynecology and Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.

Cystic fibrosis (CF) is a molecularly heterogeneous disorder that will probably be the prototype for population-based screening and, de facto, will be applied to pregnant women. In October 2001, ACMG/ACOG jointly proposed a codified approach for CF screening using a specified panel of 25 mutations. Carrier screening would be offered to Caucasian people of Northern European or Ashkenazi Jewish descent but would only be made available to those of other ethnic backgrounds. How practical have these logical but perhaps complex recommendations been? **PURPOSE:** Assess acceptance and identify problems practicing obstetricians encounter in adhering to these guidelines. **METHODS:** Our pilot study has begun within our primary medical school hospitals, after which we will extend to the broader base of referral physicians. An 18-item questionnaire inquired about the demographics and ethnic composition of each practice, manner in which screening was offered, and impressions regarding the practicality of guidelines. **RESULTS:** Even in this pilot study, a trend is apparent. The first 24 obstetricians surveyed were significantly discrepant from the ACOG/ACMG guidelines, especially concerning offering vs. making available. Only 17.4% of obstetricians followed the guidelines exactly. Most (65.2%) concluded the guidelines impractical or unclear; those practices containing a higher percentage of Caucasian/Jewish patients were more likely to view the recommendations as impractical. Physician gender, age, and practice size did not seem to alter feelings of practicality or adherence to the guidelines. **CONCLUSIONS:** This pilot study suggests a need for clarifying the ACOG/ACMG CF screening guidelines. This situation contrasts with experience initially encountered with the introduction of maternal serum analyte screening; recommended protocols for that purpose were conscientiously followed. This permitted knowledge concerning how to handle abnormal findings to be gained after a practitioner actually had his/her first abnormal test result. Different educational strategies may be needed for CF screening.

A decline in the incidence of Genetic Blood diseases in Bahrain. *S.S. Al Arrayed¹, A.Y Hamza³, D.K Shome², B. Sultan⁴, J.P Bapat².* 1) Genetic Dept, Salmaniya Medical Ctr, Manama, Bahrain; 2) Pathology department, Salmaniya medical complex, Manama, Bahrain; 3) Ministry of Health, Bahrain; 4) Maternity Nursing section. Salmaniya medical complex, Manama, Bahrain.

Genetic blood diseases are frequent in Bahrain. Previous neonatal screening done in 1985, showed that the incidence of Sickle cell disease (SCD) 2.1%, Sickle cell trait (SCT) 11%. Since then the ministry of health instituted a campaign for the prevention of these disorders. Measures included antenatal screening, student screening premarital counseling and newborn screening with health education and raising awareness among the people in the country. The incidence has been falling gradually during the years. A premarital study done in 1995, showed SCD incidence of 1.6%. Student screening done in 1999 and 2001 showed 1.3%, and 1% respectively. The Objective: to update the national data on the incidence of sickle cell disease, and compare it with previous statistics. This will help to design a future prevention program. Method: This is a collaborative project between Ministry of Health, WHO, and Bahrain Hereditary anemia society. Target Group: All Bahraini newborns delivered in the ministry of health maternity hospitals during February, to April 2002. This included 80-90% of all Bahraini newborns during that period. Types of tests: High performance liquid chromatography (Bio-Rad Variant) was used to analyze cord blood samples. Questionnaire was filled for each newborn. The form included demographic data, parental age group, and consanguinity. A coded computer program was used, data was analyzed. Results: 2000 Bahraini newborns constituted the study population. Only 17 babies were affected with SCD, with an incidence of 0.8%. 260 newborns (13%) carry the trait, the rate of 0.8 per cent represent a significant falls in the incidence (60%). report on paternal age, and consanguinity will be presented. Conclusion: Bahrain has, for the first time, recorded a less than one per cent birth rate of babies with sickle cell disease. If this trend continues, Bahrain could soon become the first country in the Gulf region to completely eliminate this disease.

“A Tale of Two SNPs” in routine genetic testing for cystic fibrosis mutations. *Y. Yang, R. Kornreich, L. Edelmann.*
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More than 1,000 mutations that cause cystic fibrosis (CF) have been described in the *CFTR* gene and hundreds of sequence variations, mainly SNPs, have been identified that either do not alter the amino acid sequence or introduce apparent benign changes. The frequencies of many of these changes, their potential cumulative effects or effects with known mutations have not been widely studied. SNPs can also interfere with PCR analyses. In our laboratory, genetic testing for CF is performed by multiplex PCR followed by ASOH, often employing previously reported primer pairs. During routine genetic testing, we discovered that two known SNPs, located on the distal primer of the exon 10 amplicon, were interfering with PCR amplification. The first SNP, 1713G>A, was identified in a delF508 carrier that appeared homozygous for the mutation. Sequencing revealed the presence of 1713G>A in trans with the delF508 mutation. This SNP coincides with the last nucleotide of the distal exon 10 primer and when present, disrupts amplification. The second SNP, 1716A>G, in the fourth position from the 3' end of the distal exon 10 primer, was also identified in a delF508 carrier. The presence of this SNP reduces amplification from the allele. We also identified this SNP in trans with delF508 in two males with infertility that were negative for the intron 8, 5T variant and are currently investigating whether this SNP, which occurs at the last position of exon 10, has any effect on splicing. We determined the frequencies of these two SNPs in 100 African American, Ashkenazi Jewish and European individuals by ASOH. We found a frequency of 7.7% for the 1713/1716 GG allele combination among African Americans and a frequency of 8.1% for the AA allele combination among individuals of European ancestry. The remainder of individuals in these two populations and all of the Ashkenazi Jewish individuals had the AG combination. We did not identify any individuals with the GA allele combination. The results indicate that these two SNPs may interfere with CF analyses in some individuals and exemplify the need to consistently examine PCR primers and probes for newly described polymorphisms.

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A novel PCR approach for detection of the common NEMO (IKBKG) rearrangement in Incontinentia Pigmenti.

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Incontinentia Pigmenti (IP, MIM 308300) is a rare X-linked dominant genodermatosis usually lethal in males during the prenatal period. Prior to identification of the IP gene (NEMO for NFkB-essential modulator), the diagnosis of mild clinical forms and genetic counseling were based on the study of the X-inactivation pattern in leukocytes, since extreme skewing of X inactivation is present in over 98 % of IP carrier females. However, X inactivation skewing is also observed in normal individuals (1%) and increases with age, so that this test lacks specificity. Recent identification of the NEMO gene enabled to show that over 80% of IP cases are accounted for by a large-scale deletion encompassing NEMO exons 4 to 10. A diagnostic procedure of this rearrangement, based on long-range PCR, has been reported (Smahi, *Nature*, 2000;405,466). However, this test was hampered by the large size of the amplification product, and additionally could not discriminate between the gene (NEMO) and pseudogene (DNEMO) deletions. We have devised a novel test based upon multiplex PCR that has proven to be NEMO-specific, robust, and reproducible. Implementation of this method markedly facilitates the detection of the common NEMO rearrangement both for postnatal and prenatal diagnosis purposes.

Development and evaluation of a model genetics screening program for multiple genetic disorders in Australian multicultural high school students: 1995-2001. *K. Barlow-Stewart¹, L. Burnett^{2,3}, A. Proos³, F. Huq¹, A. Gason⁴, M. Delatycki⁴, G. Turner⁵, H. Aizenberg⁶.* 1) Centre for Genetics Education, Royal North Shore Hospital, Sydney, Australia; 2) Pacific Laboratory Medicine Services, Northern Sydney Area Health Service, Australia; 3) Kolling Institute of Medical Research, RNSH, Sydney, Australia; 4) Genetic Health Services Victoria, Murdoch Childrens Research Institute, Melbourne, Australia; 5) Hunter Genetics, Waratah, Australia; 6) Institute of Community Genetics, Sydney, Australia.

From 1995-2000, 5,272 subjects in 20 Senior High Schools in two Australian States were offered education and the opportunity to voluntarily participate in cohort carrier genetic screening for up to three autosomal disorders relevant to the student population: Tay-Sachs disease, cystic fibrosis and thalassaemia. **METHODS:** Using de-identified coding, students were serially surveyed to measure changes in knowledge, attitude and concern about genetic screening prior to education, after education but before testing, twelve months after testing, regardless of their testing choice and 3-6 years post-testing using a mail survey. **RESULTS:** Informed choice was achieved when education was delivered in group-delivery mode combined with on-site access to testing, but not without on-site education, and also not when on-site education was provided without opportunistic access to testing. Informed choice was independent of gender, pre-existing knowledge, urbanisation, academic achievement, ethnicity and socio-demographic status. Extension of testing from one to multiple genetic disorders did not impact on informed choice or uptake. Maximal levels of uptake were achieved utilising novel result reporting systems involving holding test results in escrow until needed. Uptake levels were also maximised if genetic testing was performed without venepuncture. Long term follow-up of students showed high retention of knowledge, intended positive utility and that no harm had been done by offering testing to high school students. **CONCLUSIONS:** Cohort community genetic screening of senior high school students for multiple genetic disorders is feasible, provided certain key success factors are addressed.

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The CRMGEN Project: A New Reference Material for Hereditary Haemochromatosis Testing. *D.E. Barton, E. Donohoe for The CRMGEN Consortium.* National Centre for Medical Genetics and Department of Medical Genetics, University College Dublin, Our Lady's Hospital, Dublin, Ireland.

The use of appropriate Reference Materials (RMs) to validate test equipment or testing methods is an important part of any analytical testing system. Certified reference materials (CRMs) are RMs whose characteristics have been fully documented and validated. Currently, no CRMs are available for genetic testing. The CRMGEN project is a ten-centre collaboration funded by the European Commission's Measurement and Testing program (Contract G6RD-CT-2001-00581). We are developing reference measurement systems and producing CRMs for molecular genetic tests. Prototype RMs will be developed for a wide range of tests. These prototype RMs, developed in one of 4 genetics centres, will be validated in 7 other centres before extensive field trials. The knowledge gained in this process will be used to develop guidelines for the production of CRMs for any genetic test. Special emphasis will be given to the *commutability* of the candidate RMs, i.e. their ability to perform under a wide range of test protocols and conditions.

We have used the polymerase chain reaction (PCR) to produce prototype RMs for the common mutations involved in hereditary haemochromatosis (H63D & C282Y). Working from a 3kb master template, we have produced RMs for the individual mutations and for both together. The RMs have been tested in the Dublin laboratory, and are now undergoing testing in the laboratories of the other CRMGEN partners. As the RM production process requires these PCR products to be brought back into pre-PCR areas, serial dilutions are carried out for each PCR product, in order both to minimise the risk of contamination in second-round PCR reactions and to negate the need for scale-up in the production phase of the project. Strategies for decontamination of PCR reaction tubes and racks were also investigated, to allow future safe handling of the final product. It was found that HCl was ineffective (up to 4M), but the use of a 1/10 dilution of sodium hypochlorite eliminated all contaminating PCR products.

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Genetic cancer risk assessment (GCRA) in an underserved setting: Survey of needs, knowledge and concerns. *R. Nedelcu¹, K.R. Blazer¹, S. Sand¹, C. Arbayo¹, S. Fuentes², G.C. Uman³, J.N. Weitzel¹.* 1) Clinical Cancer Genetics, City of Hope Cancer Ctr, Duarte CA; 2) QueensCare Foundation (QC), L.A. CA; 3) Vital Research, L.A. CA.

GCRA is an emerging standard of care to identify hereditary cancer risk and target appropriate cancer prevention and early detection measures, accessible primarily to insured populations. We report results from a survey exploring knowledge, risk perceptions and barriers to cancer screening conducted prior to initiating grant-funded GCRA services in the setting of QC, which provides healthcare for families at less than 200% of the poverty level. A survey designed for grade 4-5 literacy, translated into Spanish and Korean was distributed to sequential patients. 137 surveys were analyzed. Demographics: mean age of 42; 71% Hispanic; 74% female; 70% elementary or high school education; 28% had a history of smoking cigarettes; males were twice as likely to have smoked than females ($p < .01$); 35% had a personal and/or family history of cancer; 52% had none of these risk factors. Only a minority articulated their perception of personal cancer risk. Less than half correctly answered knowledge questions about hereditary cancer. Only 37% adhered to recommended cancer screening guidelines. Over half had no insurance, with females significantly less likely to have it ($p < 0.05$). Overall, respondents were very interested in learning more about their own cancer risk and were willing to invest the time to do so. About 50% were likely at higher risk for cancer than the general population. The most frequent perceived risk factors and causes of cancer after environment and tobacco were family history and genetics, while fatalistic views of cancer were among the least identified factors. This population has increased risk for late diagnosis of cancer due to less cancer screening. Most lack insurance coverage, but economic realities did not obviate cancer concerns. Most cancer screening information was obtained from television, boding well for acceptance of telemedicine as a modality for cost-efficient GCRA-greater access could have a dramatic positive impact on patients, families and the community.

Haemochromatosis-associated HFE genotypes in English blood donors: age-related frequency and phenotypic expression. A.S. Rigby¹, V. Chambers¹, L. Sutherland¹, K. Palmer¹, A. Dalton¹, R. Sokol², R. Pollitt¹, M.S. Tanner¹, D. Gleeson³. 1) Sheffield Children's Hosp, Univ Sheffield, Sheffield, England; 2) Trent Regional Blood Transfusion Service, Sheffield, UK; 3) Liver Unit, Sheffield Teaching Hospitals, Sheffield, UK.

There is limited data on the frequency and phenotypic expression of the haemochromatosis-associated mutations C282Y and H63D in healthy people. We studied 6261 randomly selected English male blood donors (≤ 4 units donated previously). Genotyping was performed in a random sample of subjects with wild/wild and wild/H63D genotypes and in most subjects with other genotypes. The overall frequency of the C282Y/C282Y genotype was 0.3%, C282Y/H63D 2.0%, H63D/H63D 2.0%, wild/H63D 21.7%, wild/C282Y 10.4%, and wild/wild 63.4%. Genotype distribution was within H/W equilibrium within each age band. Wild/C282Y frequency fell from 11.7% in subjects < 30 years to 8.2% in subjects > 50 years ($p < 0.005$). No such trend was seen for C282Y/C282Y or C282Y/H63D genotypes. In C282Y/C282Y subjects, serum iron saturation was 63 (15-80)%; median (range) serum ferritin was 247 (60-2449) $\mu\text{g/L}$ and exceeded $> 500 \mu\text{g/L}$ in only two of 18 subjects. C282Y and H63D heterozygotes also had higher serum iron and lower unsaturated iron binding concentration than did wild/wild subjects. For wild/wild, wild/H63D and wild/C282Y and H63D/H63D genotypes, serum ferritin rose with increasing age and fell with increasing donation number. This fall was greater in wild/wild subjects than in other genotypes. In conclusion, C282Y homozygosity shows limited phenotypic expression and no trend towards age-related attrition. C282Y and H63D heterozygosity are associated with minor changes in serum iron indices and a smaller fall in serum ferritin with repeated blood donation in wild/wild subjects suggesting that both these mutations protect against iron deficiency.

Accountability in Diagnostic Molecular Genetic Testing: Europe. *S.J. Patton¹, R. Elles¹, S. Stenhouse², D. Barton³, C. Mueller⁴, M. Losekoot⁵, B. Bakker⁵, M. Goossens⁶, E. Dequeker⁷, J.J. Cassiman⁷ and The European Molecular Genetics Quality Network (EMQN).* 1) European Molecular Genetics Quality Network (EMQN), c/o National Genetics Reference Laboratory, Manchester, UK; 2) Northern Genetics Knowledge Park, Newcastle-on-Tyne, UK; 3) National Centre for Medical Genetics, Dublin, Ireland; 4) University of Wuerzburg, Germany; 5) Leiden University Medical Centre, The Netherlands; 6) Hospital Henri Mondor, Creteil, France; 7) University of Leuven, Belgium.

Diagnostic molecular genetic testing is fast becoming a routine and important part of laboratory medicine. External Quality Assessment (EQA), or Proficiency testing, is one approach to quantifying laboratory performance. The European Molecular Genetics Quality Network (EMQN) was funded to raise and maintain the quality of diagnostic molecular genetic testing in Europe. In the 5 years since the network started, the EMQN has provided thirty-five EQA schemes for twelve different inherited genetic disorders. Each scheme tests the ability of laboratories to interpret data in the light of clinical information supplied with a referral, and to produce a clear and accurate report. Laboratories from all European Union countries have participated in these exercises. The structure and development of the schemes will be described. In 2001, the schemes evaluated 315 returns from laboratories, a 33% increase on 2000. The schemes can identify critical points in the pre-analytical, analytical and post-analytical processing of diagnostic cases. The results presented show that the majority of laboratories conform to best practice guidelines, have a high level of analytical and interpretative performance, and suggest that regular participation in EQA has a significant effect towards improving the standards of diagnostic molecular genetic testing in Europe. The accountability provided by participation in such EQA schemes gives patients confidence in the results of molecular genetic testing.

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Monitoring the clinical response of mucopolysaccharidosis diseases to therapy using the MPS Test™. *C.B.*

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The clinical response of mucopolysaccharidosis diseases to current therapies (i.e., bone marrow transplantation) and future treatments (e.g., enzyme replacement therapy, gene therapy) can be monitored by measuring urine glycosaminoglycan (GAG) levels. A pattern of decreasing GAG/creatinine ratio is a surrogate marker of clinical efficacy. Zebraic's MPS Test™ allows one to send urine specimens for rapid measurement of the GAG/creatinine ratio by an automated method. For the MPS Test™, urine specimens are easily obtained by non-professional personnel (e.g., parents) from patients of any age, including infants. Specimens are easily sent through the mail without the cost or inconvenience that is required to send frozen urine specimens to other laboratories to measure GAG/creatinine by conventional methods. The availability of the MPS Test™ thus enables frequent, rapid and standardized monitoring of therapy. Importantly, every patient and healthcare provider can have access to rapid testing through a central laboratory. The broader availability of the MPS Test™ also facilitates future progress toward programs for early, presymptomatic screening of large populations to identify those individuals who are affected by mucopolysaccharidosis diseases.

Myotonic Dystrophy Type 2 (DM2) Diagnostic Testing: Results of the First Several Months of Testing in a Clinical Reference Laboratory. *J.G. Jones, S.K. Allen, L.A. Hayes, C.A. Titone, W.K. Seltzer.* Athena Diagnostics, Inc, Worcester, MA.

Myotonic Dystrophy Type 2 is associated with a CCTG repeat expansion located in intron 1 of the zinc finger protein 9 (*ZNF9*) gene. Diagnostic testing for the disease-causing expansion is challenging due to the large size (mean \gg 5000, maximum $>$ 11,000 repeats) and instability of expanded alleles. The diagnostic test involves 3 components. A PCR reaction across the repeat identifies specimens that are heterozygous for normal alleles. Expanded alleles (often 20 to $>$ 44 kb in size) cannot be amplified in this reaction, so samples that appear homozygous in this initial PCR are tested on a genomic Southern blot and PCR-based repeat assay. On genomic Southern blots, the instability of expanded alleles often leads to diffuse weak signals that span a broad size range and/or exhibit multiple band sizes. In order to maximize detection sensitivity, a DM2 repeat assay was developed by Ranum et al. (*Neurology* 58: Suppl:A317, 2002) to detect PCR products that extend partially across a long repeat region. In the repeat assay, a primer with the DM2 repeat sequence on the 3' end hybridizes every 4 base pairs along an expanded repeat to generate a broad size range of PCR products (300 to $>$ 1000 bp) that are otherwise completely absent when only normal alleles are present. Evaluation of the repeat assay PCR products by capillary electrophoresis is fast and provides high resolution, but the signals from expanded alleles gradually fade as PCR product sizes exceed about 400 bp. Detection of the same PCR products by Southern blotting and hybridization with a secondary DM2-specific oligo reveals products of much larger size ($>$ 1000 bp) and simultaneously confirms DM2 specificity. Clinical use of the diagnostic assay at Athena Diagnostics, Inc. began in May 2002. We present a summary of results on specimens submitted for clinical testing to determine (1) the number and size of expansions detected, (2) the relative proportion of DM2 to DM1 expansions, and (3) how the genomic Southern blot and repeat assay compare for detection of expanded alleles.

Program Nr: 1278 from 2002 ASHG Annual Meeting

Automated PCR Clean-Up Using the Novel Vacuum Drive Ultrafiltrations System Nucleofast. *T. Zinn, U. Schuebel, F. Hecht, M. Sieber.* Macherey-Nagel GmbH & Co. KG, D52355, Dueren,.

AUTOMATED PCR CLEAN-UP USING THE NOVEL VACUUM DRIVEN ULTRAFILTRATION SYSTEM NUCLEOFAST Thomas Zinn, Ulrich Schuebel, Frank Hecht, Manfred Sieber MACHEREY-NAGEL GmbH & Co. KG, D-52355 Dueren, Germany

Microarrays are a convenient tool for parallel analysis of gene expression. A flexible approach to investigate gene expression is the use of immobilized PCR products on microarrays. Nucleic acid purification is often the limiting step for manufacturing microarrays. The use of vacuum driven purification systems is a convenient method for automated high-throughput sample preparation.

The novel MACHEREY-NAGEL NucleoFast system for automated DNA purification provides a highly flexible purification system based on ultrafiltration. During the NucleoFast PCR 96 procedure, PCR products are transferred to a filter plate with a unique ultrafiltration membrane. Primers, non-incorporated nucleotides, and salts pass the membrane under vacuum or centrifugation while desired PCR products are retained by the membrane. Purified PCR products are resuspended and removed from the membrane. The membrane is optimized for recovery of PCR products >150 bp and shows a reproducible recovery of 50 - 95 %; for PCR fragments from 150 bp to 10 kbp. Furthermore, the permanently hydrophilized membrane shows no leaking of detergents which may have an effect on DNA spot morphology. The unique membrane allows easy removal of purified samples, even when touched with robotic tips or needles. The one-piece plate allows use under vacuum or in a centrifuge without breakage. Purification of 96 samples can be carried out in under 20 min.

In this presentation we show examples for automated PCR clean-up using NucleoFast 96 PCR on different robotic instruments. Sequencing and microarray data is presented.

Program Nr: 1279 from 2002 ASHG Annual Meeting

AUTOMATED GENOMIC DNA PURIFICATION FROM BIOLOGICAL FLUIDS. *C. Poggel, T. Zinn, U. Schuebel, F. Hecht, M. Sieber.* MACHEREY-NAGEL GmbH & Co. KG, D-52355 Dueren, Germany.

AUTOMATED GENOMIC DNA PURIFICATION FROM BIOLOGICAL FLUIDS Carsten Poggel, Thomas Zinn, Ulrich Schuebel, Frank Hecht, Manfred Sieber MACHEREY-NAGEL GmbH & Co. KG, 52355 Dueren, Germany

Clinical diagnostics require solutions for high-throughput DNA template preparation. MACHEREY-NAGEL has developed a method for fully-automated purification of up to 10 g of genomic DNA from up to 200 l whole blood or other biological fluids, starting with the blood tube and ending with highly pure genomic DNA in a 96-well format.

The Robot 96 Blood kit of MACHEREY-NAGEL adapted for use on all common liquid handling instruments provides a highly flexible purification system to the user, and ensures high reproducibility of DNA yield and purity. EDTA, citrate or heparine treated blood or frozen blood can be used. Genomic DNA purification is achieved after cell lysis and adsorption of nucleic acids in the presence of chaotropic salts to a DNA binding matrix. The proteinase K treatment is sufficient at room temperature. A processing under centrifugation is not required. Contaminants are removed by subsequent washing steps. Finally, highly pure genomic DNA (A_{260}/A_{280} : 1.8-1.9) is eluted from the matrix with low-salt Tris-buffer and can be used for downstream applications like PCR, TaqMan, Light Cycler, Southern Blot, and restriction analysis directly. Genomic DNA can be isolated with MACHEREY-NAGELs Robot 96 Blood kit in a 96-well format and even in multiples of 96. The system is free of cross-contamination, as shown by PCR. Integration of a gripper and shaker tool allows the fully automated nucleic acid purification without manual interactions.

Development of an inner ear cDNA microarray. *A.B.S. Giersch^{1,3}, K. Lyall¹, S. Hamaker¹, B.J. Quade^{1,3}, C.C. Morton^{1,2,3}.* 1) Department of Pathology, Brigham & Women's Hospital, Boston, MA; 2) Department of Obstetrics, Gynecology, and Reproductive Biology, Brigham & Women's Hospital, Boston, MA; 3) Harvard Medical School, Boston, MA.

A variety of techniques are available for detecting gene expression levels, including sequencing of cDNA libraries and analysis of Northern blots. Both techniques have been used by our research team to study individual mRNA abundance in the human inner ear. However, both techniques are limited by their inability to look at multiple messages simultaneously and under a variety of conditions.

In order to analyze transcriptional profiles of thousands of cochlear mRNAs simultaneously, we are developing a human and mouse inner ear cDNA microarray. None of the cDNA microarrays or oligonucleotide chips that are commercially available contain a collection of human and mouse cochlear ESTs. Over the past several years, more than 15,000 human cochlear ESTs have been created from the Morton human fetal cochlear cDNA library and deposited in GenBank. Over 9,000 ESTs have been generated from the mouse organ of Corti cDNA library created in the Kachar lab at NIDCD/NIH. At least one copy of each gene represented among all the human cochlear and mouse organ of Corti cDNAs will be included on the final microarray. Also included will be at least one cDNA of each known deafness gene, as well as appropriate controls. In total, there will be over 5,400 mouse organ of Corti genes and over 4,400 human cochlear expressed genes present. Up to 10% of these transcripts may be cochlear specific. We will present an update of the progress of the cochlear cDNA microarray development and preliminary data on hybridization results using a variety of human and mouse tissues.

This work is supported by NIH/NIDCD grant DC03402 to CCM and an NOHR research award to ABSG.

Modeling and Identification of Genetic Networks. *M. Xiong.* Human Gen Ctr/Houston HSC, Univ Texas, Houston, TX.

Modern biology will increasingly require quantitative analysis of the complex behavior of cellular systems and biochemical pathway. The identification and modeling of large and complex genetic networks (or pathways) are important steps toward this goal. The systematic approach provided by statistical and computational modeling and analysis has become central to unraveling genetic networks underlying biological processes. The widely used methods for construction of genetic networks have been a bottom-up approach and a top-down approach. In a top-down approach the models which can reproduce the data are constructed based on the variables measured in the whole system. This approach is gaining increased interests due to microarray technology and large-scale gene expression data. Due to the complexity of the genetic networks and experimental limitation, to precisely identify the genetic networks, which describe the direct activation and inhibition of the genes, is difficult to achieve. In this report, we present a modified concept of genetic networks which describe the quantitative relationships between genes in a broad sense and develop a mathematic framework for their modeling and identification. We provide (1) mathematic representation of genetic networks, (2) statistical methods for estimation and testing of the parameters in the models, (3) probabilistic criteria for assessments how well the models of the genetic networks explain observed data and (4) the optimization procedures for searching structure of the genetic networks. The proposed model and computational algorithms will be applied to several real data sets. The preliminary results show that the reconstructed genetic networks are consistent with the experiments.

Discovering of human cancer-related genes by EST cluster-sorting approach. *A.V. Baranova¹, D.V. Lobashev¹, D.V. Ivanov¹, L.L. Kroukovskaya², N.K. Yankovsky¹, A.P. Kozlov².* 1) Genome Analysis Lab, Vavilov Inst General Genetics, Moscow, GSP-1, Russia; 2) Biomedical center, Research Institute of Pure Biochemicals, St.Peterburgh, Russia.

Comparing patterns of gene expression in different cell lines and tissues has important applications for a variety of biological problems. Developing a computer-based procedures for subtraction of different EST pools instead of traditional experimental approaches is an attractive way to compare expression profiles. We have developed a program called HSAlyst to classify data from original dbEST and UNIGENE databases in a table form. HSAlyst software is able to arrange EST data according to any given parameter, e.g. tissue type or the number of ESTs contained in a cluster. We have tried to implement CDD approach to the searching of the human tumour-specific genes which can serve as a putative tumor markers. We performed a differential displaying of all available tumour libraries against all available normal libraries instead of pairwise comparison of each tumour and corresponding normal tissue. A comparison between the expected and observed tumor-derived EST contents shows that found tumor-related clusters are not accidental but represent a natural phenomenon. Totally 195 tumour-related clusters were found. 97 of them do not contain easily recognized open reading frames. They may be considered as an evidence in favor of the expression of newly evolved DNA sequences in tumour cells or as a manifestation of the phenomenon of the background or illegitimate gene expression, which may be enhanced in tumour cells due to deregulation of the house-keeping processes. Other 98 clusters are encode protein that can have tumor-related function. At least six of them represent well-established tumor markers, e.g. MAGEs and cancer-testis antigens. We have probed a subset of EST clusters found by HSAlyst software by both confirmatory RT-PCR and Northern experiments. The results are reassuring as mRNA corresponding to one of the probed clusters, Hs. 133294, shows an expression in four different tumour cell lines but in none of the 16 normal tissues included in MTN blot.

Analysis of the human and mouse full-length cDNA clone sets generated by the NIH Mammalian Gene

Collection. *L.H. Grouse*¹, *MGC Project Team*². 1) National Cancer Institute, NIH, Bethesda, MD; 2) Multi-Institution NIH Supported Project.

The NIH Mammalian Gene Collection (MGC) was launched two years ago with the goal of identifying and sequencing a full open reading frame (ORF) clone for each human and mouse gene. To support this goal the MGC has produced cDNA libraries from more than 100 human cell lines and tissues and 30 mouse tissues. Standard, normalization and size-selected methods are being used to produce libraries enriched for full-length clones. Initially, several hundred 5' ESTs from each library are generated and assessed to estimate the fraction of full-ORF clones and gene diversity within a library. For libraries that have a high percentage of full-ORF clones, an additional 10,000 5' ESTs are then generated. A set of algorithms, developed for the MGC, is used to analyze these ESTs for the presence of the 5' most end of an ORF. This analysis includes comparison of the MGC ESTs to the sequences of known or predicted proteins and intrinsic properties that indicate transitions between coding and non-coding sequences. Putative full-ORF clones are then sequenced to high quality and analyzed for the presence of complete ORFs. To date, approximately 65 % of the sequenced clones were found to have complete ORFs. Currently the MGC has generated approximately 8,500 human and 6,500 mouse verified full-length clones and additional putative full-ORF clones have been selected for full-insert sequencing. A list of the full-length verified genes, as well as a list of MGC libraries can be viewed on the MGC web site (<http://mgc.nci.nih.gov>.) Analysis of 5,602 of the human verified MGC full-ORF clones, revealed that for 856 of these genes, the MGC was first to provide the complete gene sequence and full-ORF clone. All full-ORF sequences and clones generated by the MGC are available to the research community through public molecular databases and clone distribution networks.

Program Nr: 1284 from 2002 ASHG Annual Meeting

The Skeletal Gene Database: A comprehensive resource for skeletal gene information. *C.A. Francomano, N.C. Ho, S. Park, D. Dudekula.* Laboratory of Genetics, National Institute on Aging, Baltimore, MD.

The Skeletal Gene Database (SGD) has been expanded to include a comprehensive catalogue of all genes and expressed sequence tags (ESTs) from skeletal tissues in the NCBI databases. A total of approximately 80,000 ESTs from 301 libraries derived from cartilage, bone, bone marrow, synovium, chondrosarcoma and osteosarcoma are now included. The database is set up to search by NCBI accession number, chromosome location or by gene name. The preliminary search pulls up a screen offering links for the relevant gene, EST or chromosomal region to GenBank, UniGene, LocusLink, Online Mendelian Inheritance in Man (OMIM) and PubMed, allowing quick access to relevant genomic resources on the internet. Users may link directly to the SNP, SwissProt, gene ontology, EC and human chromosome mapping data sets for specific information about their gene or EST of interest. The Skeletal Gene Database may be accessed at the URL <http://sgd.nia.nih.gov> after August 1, 2002.

A functional genomics approach of multigenic Hirschsprung disease: comparative transcriptome expression of predicted and known genes in candidate intervals. *M. Clement-Ziza¹, T. Attié-Bittach¹, J. Amiel¹, J. Audollent¹, M. Vekmans¹, A. Chakravarti², A. Munnich¹, S. Lyonnet¹, C. Besmond¹.* 1) INSERM U393, Hopital Necker Enfants Malades, Paris, FRANCE; 2) Departement of Genetics, University Hospitals of Cleveland, Cleveland, Ohio 44106, USA.

Hirschsprung disease (HSCR), the most common hereditary cause of intestinal obstruction (1/5000), shows considerable variation and complex inheritance. HSCR is ascribed to an abnormal migration of the neural crest cells leading to the absence of enteric nervous system in the colon. The mutations identified in the RET, GDNF, EDNRB and SOX10 genes fail to explain the transmission of the most common form of the disease: short-segment HSCR. Recently, a large non-parametric study led to the identification of three susceptibility loci on 3p21, 10q11 and 19q12 respectively (Nat Genet. 2002; 31:89-93). RET, which maps to chromosome 10q11.2, is the major disease gene and accounts for largely 50% of mutations.

In order to identify the genes located at these loci and involved in the disease, we carried out a comparative transcriptome expression study of the genes included in the physical intervals defined by our non-parametric study. Known and predicted cDNA sequences, along with their structural and functional annotations (when available), were extracted from Ensembl and NCBI databases. These data were ordered according to objective criteria such as number of exons, location within the interval or functional annotation. Specific 50-mer oligonucleotides from the cDNA sequence of 700 genes were designed and spotted onto an aldehyde-coated slide in order to make an expression microarray.

We analysed the differential expression of these genes in HSCR vs normal colon, as well as in fetal gut tissues before vs after neural crest migration. The differentially expressed genes are candidate genes in HSCR.

Computational Analysis of Pharmacogenetic Variations. *A. Brower, F. Cao, B. Hurwitz, D. Machmeier, I. Trifunovich, B. Neri, L. Fors, V. Lyamichev, A. Lukowiak.* Bioinformatics, Third Wave Technologies, Madison, WI.

Pharmacogenetics provides the experimental framework for dissecting the effects of genetic variation on human drug response. The application of genetic variation profiles promises to foster the application of personalized medicine and to avoid the occurrence of adverse drug responses. Identifying the sequence variations that are involved in drug response is an important step in designing meaningful pharmacogenetic studies.

To date there are over 3 million sequence variations have been deposited in public databases as a result of the Human Genome Project, the SNP Consortium (TSC), the Japanese Millenium Project, and several other SNP discovery projects. Only a fraction of these variants may be important in the genetics of drug response. The current challenge is to identify a collection of variations for use in drug discovery, clinical trials, and association studies. We present a computational method that markedly accelerates these types of pharmacogenetic studies by integrating sequence variation with gene annotation & literature mining. Our approach uses novel algorithms applied to a curated set of Human genes and results in the annotation of over 50,000 variations in 1003 genes that are thought to be involved in drug response. The sequence variations were derived from various public sources such as NCBI's SNP database (dbSNP) & Online Mendelian Inheritance in Man (OMIM), Japan's JSNP, gene specific web resources, medical literature, as well as proprietary sources. All SNPs were mapped to the April 2002 hg11 human genome assembly (NCBI Build 29). Based on the mapped positions, SNPs were grouped into several functional locations that included locus, gene, CDS, intron, exon, and splice sites. The computational program facilitates the study design to focus on genetic variations that are predicted to make a functional impact on the protein product and/or on covering the genic region to capture haplotype information. This set has been used to populate panels of Invader® SNP assays targeted to address specific questions related to pharmacogenetics.

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Software Agent to Automatically Update Genomic Information. *D. Chen*^{1,2}, *H.F. Orthner*¹, *S.M. Sell*². 1) Health Informatics Program, Department of Health Services Administration, School of Health Related Professions. University of Alabama at Birmingham, Birmingham, AL, USA; 2) Genetic Core Facility, NIDDK-Clinical Nutrition Research Center, Department of Nutrition Sciences. School of Health Related Professions, University of Alabama at Birmingham, AL, USA.

The rapid growth of genomic information, especially genetic maps available from NCBI and various online sources raises questions about how to update them in a timely fashion. The objective of this study is to automatically retrieve, store and visualize the updated genomic information based on user preferences on genes, chromosomal locations and preferred sources such as National Center for Biotechnology Information (NCBI) MapViewer, Sanger Center, UC Santa Cruz (UCSC) Genome Browser and the Medline. The related topics or databases are visualized over the Web through the wired or wireless network. In our case, we focused on human chromosome 20 and mouse chromosome 2. We have developed a Medline searching agent called EZbutton that retrieves the above information in a predetermined schedule. The EZbutton was built on a personalized domain-specific vocabulary (PDSV) database that stores the user preferences. Web-based user and administrator interfaces were created to facilitate the user-friendly and individualized presentation and user account management. The preliminary results based on user satisfaction survey showed that the individualized information retrieval increased both efficiency and relevancy. We hypothesize that the Medline agent (EZbutton) has potential to be an efficient tool for genomic research scientists to get relevant information from various sources especially from the NCBI GenBank. (This project has been funded in part with funds from the NIH grants F38LM07185, P30DK056336 and R21HD040788).

Comparative analysis of SNPs to define a putative long-range control element. *Y. Chen, S.M. Sell.* Dept. of Nutrition Sci., Univ. of Alabama at Birmingham, Birmingham, AL.

Over 2.8 million SNPs have been submitted to the NCBI SNPs database (dbSNP). However, many more SNPs are still to be discovered, and the functions of most SNPs need to be identified. Conserved noncoding sequences (CNS) could play an important role in genetic control processes, although the precise role and mechanism are also poorly understood. VISTA (Visualization Tools for Alignment) is a set of tools for comparative genomics. It was designed to visualize long sequence alignments of DNA from two or more species with annotation information. Here we present an example. We analyzed a 100kb block of sequence upstream of a candidate gene for insulin resistance. The results show that most CNS peaks (25/32) contain SNPs. The distribution of SNPs is similar to the distribution of CNSs. We also analyzed a 10 kb sequence (30 kb-40kb upstream of the gene). For most CNS peaks (5/6), there exist SNPs. Also most SNPs (28 out of 30) are in a CNS region or within 500b of a CNS. Certain of these SNPs could play a very important role in the regulation of the candidate gene. We then checked the sequence of CNS for SNPs in our study population. Some new SNPs (UABSNP58, UABSNP57) in this area were verified. Among these SNPs, UABSNP58 has been shown to be associated with insulin resistance. This research can provide some background information for development of a tool to predict the existence and function of disease SNPs. This work was supported by funds from NIH grant DK056336.

	100kb upstream	30-40kb upstream
# SNPs	137	30
# CNS	37	6
% identity	43	38

Microarray analysis of a cell culture model of early onset dystonia linked to Torsin A mutations. *M.J. Baptista¹, C. O'Farrell², R. Ahmad¹, M.R. Cookson¹.* 1) Neurogenetics, NIA/NIH, Bethesda, MD; 2) Neurogenetics, Mayo Clinic, Jacksonville, FL, 32224.

Early-onset torsion dystonia is a movement disorder characterized by sustained muscle contractions and abnormal posture. A deletion in a conserved region of the torsinA gene, resulting in the loss of one of a pair of glutamate residues (DE302/303), has been detected in a large number of patients with chromosome 9-linked primary dystonia (DYT1). However, mutations in torsinA are not fully penetrant and some genetic heterogeneity has been noted, suggesting that additional genetic factor(s) may play a role in the development of this disease. The first aim of the current study was to use a cell culture model of torsinA over-expression to identify additional genes relevant to dystonia. The second aim was to identify genes whose expression is altered by torsinA in the hope of identifying novel markers of cellular dysfunction caused by the presence of these mutations. Clonal cell lines over-expressing either wild type torsinA or the DE302/303 mutation were generated by stable transfection of HEK293 cells. We measured torsinA expression using both RT-PCR and protein and identified several clones of both wt and DE302/303 with similar levels of expression. We then used the oligo-based U95A GeneChip (Affymetrix, Santa Clara, CA) to globally compare the expression levels of 12,500 non-redundant cDNAs and ESTs. Sixty-five probe sets were identified showing differential expression levels when compared to cells transfected with the empty vector. Of particular note are the family of heat shock proteins, and their transcription factor (hsf-2). While hsf-2 displayed a reduced expression, the expression of heat shock proteins 70 and 90 was increased approximately six fold. The microarray data will be confirmed by quantitative RT-PCR using SYBR green as a reporter. It is expected that identification of additional gene families regulated by over-expression of torsinA, both wild type and the DE302/303 mutation will provide additional information towards defining a pathway leading to dystonia.

Program Nr: 1290 from 2002 ASHG Annual Meeting

Mutation detection and candidate gene resequencing using the ABI Prism 3100-Avant Genetic Analyzer. K.

Gunning¹, R. Brugada², K. Hong², A. Oliva², K. Chansky¹, L. Joe¹, Q. Doan¹. 1) DNA Platforms Business Group, Applied Biosystems, Foster City, CA; 2) Masonic Medical Research Center, Utica, NY.

In order to determine the genes involved in autosomal dominant disease we require a flexible detection system capable of performing a variety of applications. The ABI Prism 3100-Avant Genetic Analyzer has proven to be an adaptable platform for generating data of the highest quality. We have used the instrument to generate both sequencing and fragment analysis data for our research endeavors in the area of cardiovascular disease. We have previously identified one gene responsible for Brugada syndrome on 3p, the cardiac sodium channel SCN5A. We have collected more than 200 probands and families with the disease. The analysis of segregation of new families with the locus is performed efficiently with the fragment analysis application. The analysis of the gene and mutation detection is powerfully enhanced by the higher throughput of the equipment and the availability of new software for sequencing comparison. The use of this capillary electrophoresis platform greatly reduces the degree of hands on time required and is a low maintenance system, allowing us time to perform the other tasks associated with the development of our research. Integration of SeqScape Software version 1.1 into our analysis pipeline has further advanced our research by providing a tool for data analysis. We will discuss the role of the ABI Prism 3100-Avant Genetic Analyzer, along with SeqScape Software v 1.1, in the human disease research laboratory for the purposes of mutation detection.

DNannotator: a web-based sequence annotation tool kit and its application on analyses of 13q32-33 sequence.

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Annotated sequence is the basis of positional cloning and association studies searching for susceptibility genes, and it is also important for managing sequence-related data and guiding experimental design in "wet" labs. DNannotator, a web application, was designed to address the problem of automatic, customized, high-throughput annotation on sizeable genomic region. It simplifies sequence annotation, merging of annotation data and re-annotation when sequence is updated. DNannotator takes two basic approaches to annotation: 1) BLAST match-based approach that is based on the filtered BLAST matches of annotation source and target sequences; and 2) Direct parsing of outputs of third-party software such as e-PCR and Sim4. Annotation data are generated in Genbank format, along with a feature table in plain text format, which can be imported and managed in a user-preferred database or spreadsheet. Feature-related sequence fragments can be extracted from annotated sequences for further study or for annotation transfer. DNannotator was used to do de novo annotation for 43 known full-length genes and their 561 exons, 557 primers, 431 TSC SNPs and more than 400 STSs to 3 different versions of ~ 20 Mb genomic DNA assemblies of human 13q32-33. Annotations from NCBI build 27 were transferred to NCBI build 28 to demonstrate the functionality of preserving annotations in DNannotator. The results show that DNannotator is efficient and accurate. The quality of 4 gDNA assemblies of the same region is evaluated, based on the annotation results. The comparisons reveal a potential "in-region duplication" and "flip block" problem in current public draft sequence assemblies. Results provide a preliminary evaluation of these assembled sequences of this region, and a basis for searching for a susceptibility gene of Bipolar Disorder on 13q.

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Comprehensive Resource: The Cancer Genome Anatomy Project. *L. Jia, CGAP Project Team.* Office of Cancer Genomics, NCI/NIH, Bethesda, MD20892, USA.

The National Cancer Institute implemented the Cancer Genome Anatomy Project (CGAP) to create an information infrastructure of the genes associated with cancer development, and to develop technological tools to support the analysis of the molecular profile of cancer cells and their normal counterparts. The current CGAP program comprises Tumor Gene Indices for the human and mouse (hTGI and mTGI), a Genetic Annotation Initiative (GAI) and the Cancer Chromosome Aberration Project (cCAP). The TGI and GAI, are focused toward building a catalog of annotated genes associated with cancer. The third component, cCAP, is developing resources to catalog and facilitate the molecular characterization of cancer-related chromosomal aberrations. The presentation will highlight online tools for accessing the CGAP data as well as informatics tools for in silico analysis.

A computational/functional genomics approach for the identification of positional candidate retinopathy genes.

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Grouping genes by virtue of their sequence similarity, functional association or spatiotemporal distribution is an important first step in investigating function. Given the recent identification of >30,000 human genes either by analyses of genomic sequence or derivation/assembly of ESTs, automated means of discerning gene function and association with disease are critical for the efficient processing of this large volume of data. We have designed a series of computational tools to manipulate the EST sequence database (dbEST) to predict EST clusters likely representing genes expressed exclusively or preferentially in a specific tissue. We implemented this tool by subtracting 40,000 human retinal EST sequences from a total of 1.4 million ESTs represented in over 100 tissues, and identified 925 ESTs likely to be specifically or preferentially expressed in the retina. Upon verification of the computational predictions by RT-PCR and microarray analysis with RNA from 24 adult and fetal tissues, we mapped the retinal-specific/predominant sequences in the human genome and produced a web-based searchable map of the retina transcriptome. To expedite the identification of disease-causing candidate genes, we incorporated into this map the positions of all mapped but uncloned retinopathy loci. This resource has provided positional candidates for 42/51 uncloned retinopathies and may expedite substantially the identification of disease-associated genes. More importantly, the ability to systematically group ESTs according to their predicted expression profile is likely to be an important resource for studying gene function in a wide range of tissues and physiological systems and to identify positional candidate genes for human disorders whose phenotypic manifestations are restricted to specific tissues/organs/cell types.

Evaluation of Gene Expression in Fetal Mice Molars through SAGE. *M.M. Michalec¹, M.J. Gorry², W. Wang², L. Lu², P.S. Hart¹, T.C. Hart²*. 1) Human Genetics, Univ Pittsburgh, Pittsburgh, PA; 2) Dept Oral Med/pathology, Univ Pittsburgh, Pittsburgh, PA.

Candidate gene studies of tooth defects are hampered by the fact that all gene in the development of teeth are not known. Serial analysis of gene expression (SAGE) is a robust method for global gene expression profiling. In order to catalog genes important in tooth development, a SAGE library was made using pooled fetal mice molars (14-18 days gestation) and the I-SAGE kit (Invitrogen). 5,000 clones were selected from the library for study. Of 57,477 tags sequenced, a total of 9925 unique tags were obtained (tags with hits of 2 or greater). Not surprisingly, the most highly expressed gene was amelogenin, a tooth specific gene highly expressed in enamel. Also highly expressed were collagen 1A1 and collagen 1A2 and dentin sialoprotein, all three proteins are known to occur at high amounts in tooth dentin. In addition, a large number of genes were identified that were not previously known to be expressed during tooth development. Comparison with available SAGE databases allowed the identification of several tooth-specific tags that presumably correspond to previously unknown genes. As it has become increasingly apparent that a given SAGE tag may correspond to more than one gene, we sought to verify tag identification through 3 methods: rSAGE, GLGI, and Western blot analysis. As an example, using rSAGE, we identified the highly expressed unknown TGGAAATGAC tag as myristoylated alanine rich protein kinase C substrate (Macs). This study will be useful in identifying genes involved in tooth development and patterning, as well as identifying candidate genes for genetic tooth defects, such as amelogenesis imperfecta.

Characterization of lung allograft rejection using microarray gene expression profiling. *J.D. Lande, V.J. Gimino, M.I. Hertz, R.A. King.* Medicine & Inst Human Genetics, University of Minnesota, Minneapolis, MN.

Obliterative bronchiolitis (OB) is a fibroproliferative process that blocks small airways and is the most significant limiting factor for long term survival of lung transplant recipients. Acute rejection, characterized by lymphocytic inflammation of small blood vessels and airways, is a major risk factor for chronic rejection. We are using oligonucleotide microarrays to identify reproducible patterns of gene expression that characterize acute and chronic lung rejection. Total RNA isolated from bronchoalveolar lavage (BAL) cells from lung transplant recipients (n=16) was processed and hybridized to U133A human GeneChips. Two different software applications were applied to estimate transcript abundance measurements: Affymetrix Microarray Suite version 5 (MAS 5), that calculates signal, which is applied to each array individually and can be compared if all arrays are scaled or normalized; and DChip, that integrates data from all arrays being compared for the calculation of model based expression index (MBEI), a measure of transcript abundance. The coefficients of variation ($CV = \text{standard deviation}/\text{mean}$) were calculated for both signal and MBEI. Those transcripts that were present in at least 20% (>3 of 16) of all arrays and having a $CV > 1$ were considered candidates. There were 177 candidate transcripts using MBEI to measure transcript abundance and 158 using Affymetrix signal. Of these, 97 transcripts were common between the two sets. Functional analysis of these common transcripts, using annotations from the Gene Ontology database, indicate that many of the genes expressed differentially across arrays are relevant to processes believed to be involved in the pathogenesis of acute and chronic rejection. Cluster analysis of these transcripts using Cluster (Eisen) suggest that much of the variation is due to two individuals with distinct profiles of genes that appear to be involved with immunological processes. Microarray analysis of RNA isolated from BAL samples of lung transplant recipients may be helpful in identifying genes related to lung transplant rejection.

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Genetic Control of Gene Expression: A study combining Gene expression with Genebased haplotypes in Immortalized B-cells. *B.T. Koshy, M. Kumar, A. Windemuth, K. Nandabalan, R. Judson.* Bioinformatics, Genaissance Pharmaceuticals, New Haven, CT.

Expression arrays provide a global view of transcriptional regulation. To derive meaningful biological insight about regulatory mechanisms, most published studies are limited to clustering genes with similar expression patterns. We use a novel approach that combines gene expression with gene based haplotypes to provide clues about genetic control of regulatory mechanisms in biochemical pathways known to operate in B lymphocytes. Using a statistical screening method called Ruler Finder that searches for associations between genetic markers and expression levels on a matrix of 5,500 gene-based haplotypes by 9,500 expression levels, we provide evidence for genetic control of previously recognized as well as novel regulatory elements in the NF-kB pathway. The power of our combined approach is that it provides evidence of the directionality of regulation within a pathway which cannot be deduced from expression clustering alone.

Program Nr: 1297 from 2002 ASHG Annual Meeting

Designing LIMS to bundle with high-throughput production systems for resale: Wildtype Linx™ lab tracking software and Illumina, Inc's BeadArray™ technology platform. *T.J. Stevens, J. Thompson.* Wildtype Informatics LC, San Diego, CA.

LIMS software bundled into a resalable high-throughput production system is subjected to very different demands than lab tracking software customized for a single lab's needs. Unlike a primary LIMS purchase, bundled LIMS arrives in the lab as one component in a proprietary package of SOPs, instruments, and automated liquid handling workstations. The purchasing lab's goal is to simply turn the key and start producing thousands or millions of data points as promised by the vendor.

We set out to design and build a LIMS software platform that would win over new owners of bundled high-throughput production systems, and still remain flexible enough to address the highly custom requirements of single-site LIMS projects. The result, Wildtype Linx, offers a long list of features including a high-performance Java and XML architecture, support for web browser clients, easy integration with robotic processors and other clients via XML over HTTP, sub-second response on most LIMS transactions, and few maintenance needs.

In this presentation, we evaluate how well Wildtype Linx has met its design goals in its first bundling, as a component in Illumina Inc.'s (San Diego, CA) world-leading throughput systems built on their proprietary BeadArray technology platform.

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Disordered Defensins and Perplexing Polymerases. *M.W. Wright, E.A. Bruford, R.C. Lovering, M.J. Lush, S. Povey, H.M. Wain.* HUGO Gene Nomenclature Committee, The Galton Laboratory, Department of Biology, University College London, Wolfson House, 4, Stephenson Way, London, NW1 2HE, UK. Email: nome@galton.ucl.ac.uk.

The HUGO Gene Nomenclature Committee (HGNC) strives to avoid confusion when talking about genes by creating a common nomenclature, so that each gene has its own unique symbol that everyone can recognise. This ongoing project has so far given approved symbols to almost 15,000 genes.

Many journals appreciate how important a common language is for enhanced communication, and The American Journal of Human Genetics, Annals of Human Genetics, Cytogenetic and Genome Research, Genomics, Human Mutation, Molecular Therapy, Nature Genetics and Radiation Research actively support our efforts by insisting that authors only use HGNC approved symbols. However, there is plenty of room for improvement by other genetics journals; each year several new genes are published with a variety of different names, and different genes are published with the same name. Promisingly, the need for standardisation is being recognised by the community, as more and more authors publishing in other journals contact us prior to publication, because they want their work to be readily accessible via the use of approved symbols in the public databases. To this end we edit Genew our online nomenclature database (<http://www.gene.ucl.ac.uk/nomenclature/>), and NCBI's LocusLink (<http://www.ncbi.nlm.nih.gov/LocusLink/>), and this information proliferates to many other databases.

With the help of the genetics community, we have recently resolved the very perplexing polymerases and the disordered beta defensins, along with other examples of gene confusion as we will show.

The work of the HGNC is supported by NIH contract N01-LM-9-3533 (60%) and by the UK Medical Research Council (40%). Please come and see us at the Nomenclature Booth.

Program Nr: 1299 from 2002 ASHG Annual Meeting

Bioinformatics for DHPLC. *P.D. Taylor, Y. Lui, G. Narayanaswami, M. Piff.* Dept R&D, Transgenomic, Inc, San Jose, CA.

The procedural flow for DHPLC can be described as: a)genomic context selection, b)amplicon design, c)sample preparation, d)data acquisition, e)data analysis, f)confirmation of polymorphism, g)archiving of results and finally, h)application of results. Using the data acquisition step d as the center, the steps a-c may be termed pre-processing and the steps e-h may be termed post-processing. This work describes some of the problems encountered during pre- and post-processing of many DHPLC studies and the solutions and recommendations developed. A small Microsoft SQL Server 2000 database was developed to contain the selected contexts and annotations including intron/exon boundaries. Target sequences are retrieved from the database, submitted to PCR design and the melting properties scanned for DHPLC suitability. Amplicons can be redesigned in-silico and finally added to the database along with the melting properties and PCR annealing conditions. After sample preparation and data acquisition the traces are grouped and results displayed as a scattergram. When groups containing the mutations have been confirmed by sequencing, a number of SQL stored procedures in the database assist with the correct annotation of the fragment and mutation with minimal manual intervention. There is a clear need for database tools which ease the process of SNP data entry and check for inconsistency in the information both during and after input.

Primer Design and Marker Clustering for multiplex primer extension SNP genotyping assay using statistical modeling. *A. Yuryev, J. Kuebler, M. Donaldson, K. Scott, J. Studebaker, J. Huang, M. Pohl, M. Boyce-Jacino.* Bioinformatics & Genetics, Orchid Biosciences Inc, Princeton, NJ.

The size of SNP genotyping projects is rapidly expanding as more information is gathered to prove the utility of SNP markers in association studies, linkage studies and mapping projects. Methods of reducing cost and increasing genotyping throughput are required in order to take full advantage of SNP markers in the genome studies. One strategy is to multiplex the genotyping reactions. We have successfully demonstrated multiplexing SNP-IT in groups (multiplex fold) of 12 and 24 markers. Using data collected from multiplex studies, we have developed a statistical algorithm that is used to design assay primers and group SNP markers together in a cluster. This algorithm has enabled us to improve our success rate of multiplexing (number of valid assays per cluster) from 75% to 96%. Our clustering algorithms are reminiscent of two major classes of clustering algorithms: hierarchical and non-hierarchical. The statistical model for multiplex SNP-IT reaction was developed using logistic regression analysis and a set of 23,525 SNP markers. The set had both successfully genotyped (passed) markers and failed markers. The model was trained to distinguish failed and passed markers by assigning them the failure probability score in the range of 0 to 100%. The model calculates the failure probability of individual SNP markers based on the internal marker sequence characteristics and external interference from other markers in a multiplex cluster. The combination of external statistical scores contributing to the multiplex model was chosen as minimization function for clustering algorithms. The first clustering algorithm randomly exchanges markers between clusters and accepts only those marker swaps that decrease the combined failure rate of two multiplex clusters. The second algorithm does pair-wise clustering of markers and smaller size marker clusters until the necessary cluster size (equal to multiplex fold) is reached. Based on the average failure probability, we found that random clustering performs better for most of the marker sets.

Program Nr: 1301 from 2002 ASHG Annual Meeting

Integrating Genomic Laboratory Data with Whole Genome Annotation Using Ensembl-DAS. *H. Xu, J.M. Vance, J.E. Stenger.* Center for Human Genetics, Duke University Medical Center, Durham, NC.

We have developed perl programs to integrate our laboratory data into a local version of Ensembl with the distributed annotation system (DAS) developed by Dowell, Eddy and Stein. Ensembl is open source and was set up on our local server using instructions provided at ensembl.org. The DAS database has one table to hold genomic locations for all DAS features. Each type of DAS features has one or more tables to hold the detail information. DAS features are linked to their genomic location through their IDs. There are several tables containing internal research project information and external database information. DAS features are also linked to project and external database information through their IDs. Data that we have already included are genetic linkage, variation, microarray, and serial analysis of gene expression (SAGE). They were mapped to human genome assembly through three-stage pipeline: First, database lookup located experiment features that were already mapped in public genome annotation databases. If the experiment features have known IDs, such as GenBank accessions, HUGO gene symbols, or genetic marker names, the IDs were used to query local Ensembl, NCBI, or UCSC tables to get their genomic locations. If no location can be found, those features were passed to the next stage. Next, electronic PCR located features that have PCR primers available. If the experimental features have PCR primers and PCR fragment size, they will be mapped to human genome by electronic PCR. If no location can be found, those features were passed to the next stage. Thirdly, BLAT located features that have sequences available. If the experimental features have surrounding sequence information, they will be put into a FASTA format file. Then this file will be used as query for a BLAT search of the human genome assembly. All the feature locations were put into DAS database. The feature information is parsed to put into DAS tables as well. Finally local Ensembl displays our experimental features in a contig view of the human genome.

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A comparison of software tools for comparative sequencing. *J.M. Sorenson¹, K. Gunning¹, S. Kumar¹, Q. Doan¹, P. Suri¹, R. Paul¹, A. Pradhan¹, P. Honebein², G. Denisov⁴, M. Schoppe³, A. Pronyaev³, L. Johnston-Dow¹.* 1) Applied Biosystems, Foster City, CA; 2) Systems Integration Solutions, San Francisco, CA; 3) Group Logic, Arlington, VA; 4) Paracel Inc., A Celera Business, Pasadena, CA.

The release of version 1.1 of SeqScape™ software marked the introduction of basecalling quality values and assembly-based SNP identification into a desktop comparative sequencing software tool. SeqScape™ software is compatible with Windows NT®/Windows® 2000 OS and contains fully integrated basecalling, trimming, sequence assembly, alignment, and sequence comparison tools for fast and accurate sequence comparisons and variant identification. We present here a comparison of the features, ease of use, and robustness of algorithms between SeqScape™ software and other available comparative sequencing software tools. We also critically evaluate the accuracy of analysis results from SeqScape™ software version 1.1 versus Sequencher™ software. Highlights are presented of new algorithm features in development for the next version of SeqScape™ software, including the ability to detect and identify heterozygous frameshift mutations using direct sequencing.

Use of a custom oligonucleotide microarray to study imprinting in the mouse CNS. *V.L. Buettner*¹, *M.E. Barish*², *J.A. Longmate*³, *J.R. Mann*¹, *J. Singer-Sam*¹. 1) Division of Biology, Beckman Research Institute, Duarte, CA 91010; 2) Division of Neurosciences, Beckman Research Institute, Duarte, CA 91010; 3) Department of Biostatistics, City of Hope National Medical Center, Duarte, CA 91010.

Imprinted genes play an important role in mammalian growth and development and are known to be involved in certain genetic disorders of the CNS [i.e., Prader Willi and Angelman syndromes(PW/AS)]. There is, however, no rapid assay for imprinting or for the identification of novel imprinted genes. We have developed an oligonucleotide microarray-based assay using mice carrying maternal or paternal duplications of chromosomal regions of interest. As a model system, we are analyzing RNA from brain tissues of neonates carrying only maternal or paternal copies of proximal chromosomes 7 and 15 (the T9H translocation), including the region of chromosome 7 corresponding to human PW/AS. The custom array contains probes for housekeeping genes, known imprinted genes and an additional ~300 CNS-enriched genes critical for neurotransmission and/or mapping to proximal 7 or 15. Prior to microarray analysis, we compared mat7dup and pat7dup RNA from neonatal cerebellum, cortex, prefrontal cortex, and diencephalon by use of quantitative RT-PCR to verify imprinted expression of the known imprinted genes included on the array: *Ndn*, *Snrpn*, *Zfp127*, *Ipw*, *Magel2*, *Peg3* and *Ube3a*. Comparing these RT-PCR results with those obtained by microarray analysis, we found that sensitivity of the microarray varies depending upon the brain region analyzed, an important factor being tissue-specific background values. The most abundantly expressed imprinted genes, *Ndn*, *Snrpn* and *Ube3a*, were detectable in all brain regions analyzed while genes expressed at a lower level were most easily detectable in cerebellum and cortex. We have found no evidence for imprinting of any of the genes that map to proximal chromosome 7 or 15, nor of secondary effects of the chromosomal duplications on unlinked genes involved in neurotransmission.

Analysis of the human phenome by clustering xenoscapes in *Drosophila*. E. Bier, L. Reiter. Cell and Developmental Biology, Univ. of Cal., San Diego, La Jolla, CA.

A variety of seemingly dissimilar phenotypes can result from various mutations in the same human gene or by inappropriate spatial or temporal expression of that gene. We refer to this array of phenotypes as the phenoscape of a gene and the genome wide collection of phenoscapes as the phenome. With the advent of GAL4/UAS misexpression system in the model organism *Drosophila melanogaster* it is possible to create a genome wide collection of xenoscapes, or the set of *Drosophila* loss-of-function and misexpression phenotypes generated for all human genes. Although the *Drosophila* xenoscape of a human gene may bear little if any resemblance to the phenoscape of that gene in humans, we suggest that clustering human genes based on *Drosophila* xenoscapes of normal and mutant forms of human genes should result in a grouping of human genes into coherent functional subsets, and may provide valuable information for the eventual assembly of the human phenome. The ability to screen for multiple dominant alleles of a gene of interest in *Drosophila* using the recently devised NOVA method on human transgenes should greatly increase the informational content of particular xenoscapes. This type of semantic translation between *Drosophila* and human phenoscapes can already be applied to known homologous pathways like the Notch signaling pathway and herein we discuss how it might be used to identify new genes involved in identifying cardiac disease candidate genes. We also discuss the importance of creating controlled vocabularies to define phenoscapes for comparisons between species.

Identification of differentially expressed genes during development of cardiac hypertrophy in rat by subtractive hybridization. *T. Hahn, D. Schlote, I. Hansmann, M. Schlicker.* Institut fuer Humangenetik & Medizinische Biologie, Halle (Saale), Germany.

Cardiac hypertrophy is an adaptive response to chronically increased workload. It appears to be associated with altered gene expression leading to cardiomyopathy and heart failure. To identify candidate genes contributing to the initiation or progression of cardiac hypertrophy we have screened differential gene expression in the heart of spontaneously hypertensive rats (SHR) at different stages of their development. During first weeks after birth the SHR rats are normotensive. Males first develop hypertension around 12 weeks pp and hypertrophy of the left heart ventricle around 26 weeks pp. We established a subtractive hybridization and cDNA cloning system based on cDNA selection and suppression PCR using mRNA from these stages. Subtractive hybridization using age groups 4- versus 12- as well as 12- versus 26-weeks identified 145 different cDNA clones. Screening of these clones revealed 56 cDNAs as candidates for differentially expressed genes during development to cardiac hypertrophy. Northern blot analysis of 42 cDNAs identified 16 genes to be upregulated in cardiac tissue of SHR in comparison to the wild type Wistar-Kyoto rat. In silico analysis of cDNA sequences identified several known genes which are being discussed already in the context with cardiomyopathy (g-sarcoglycan) or cardiac hypertrophy (acyl-coA dehydrogenase) which can be used as an argument in favor of the validity of our concept. In addition to these genes our analysis revealed so far 8 novel genes which are differentially expressed in the heart of SHR. Mapping of these novel genes with respect to rat chromosome segments harboring known QTLs for cardiac hypertrophy and analysis of their expression pattern might be relevant to understand processes leading to cardiac hypertrophy in rat and man.

ELUCIDATION OF PATHOGENIC GENETIC PATHWAYS IN THE HYPERTROPHIC AND FAILING HEART BY MICROARRAY TECHNOLOGY. *H. Smeets*^{1,2}, *B. Van den Bosch*^{1,2}, *S. Van der Vlies*^{1,2}, *J. Geurts*^{1,2}, *I. De Coo*³, *H. Scholte*³, *D. Lips*², *P. Doevendans*². 1) Dept Genetics & Cell Biol, Univ Maastricht, Maastricht, Netherlands; 2) CARIM, Univ Maastricht, Maastricht, The Netherlands; 3) Dept Pediatric Neurology and Biochemistry, Erasmus University, Rotterdam.

Gene expression differences between normal, hypertrophic and failing heart may provide therapeutic targets for interventions in patients. To circumvent the complexity and scarcity of human biopsies, we used inbred mice, in which Transverse Aortic Banding (TAC) was applied to induce cardiac hypertrophy, followed by heart failure. Left ventricle RNA of these mice and controls (48 hours, 1, 2, 3,6 and 8 weeks after operation) was labeled with Cy3/Cy5 (indirect amino-allyl-labeling) and hybridized to microarrays containing ~4,000 cDNA clones derived from adult and fetal mouse heart. Experiments were performed in quadruplicate, using at least 3 mice per time point. Scatter plots showed that expression levels of the majority of genes was unaltered. Known hypertrophy markers (f.e. betaMHC, ANP, SPARC, CARP) were elevated as expected. Initial results showed >2-fold altered gene expression of a number of unknown and known genes, including connective tissue growth factors, imprinted mesoderm specific embryonic genes and calcineurin-interacting proteins in the hypertrophic myocard after 7 days. Specific mitochondrial genes displayed a decrease after 42 days, when heart failure could be observed. Gene expression changes were confirmed by quantitative PCR and related to functional changes, in the latter case for example by measuring respiratory chain enzyme activities. Our next step will be to test the differentially expressed genes in human biopsy specimens of non-diseased, hypertrophic and failing myocard. The newly identified genes will be evaluated for a role in familiar hypertrophic or dilated cardiomyopathy by their map position and segregation in families.

Clinical and molecular delineation of the 22q11 deletion syndrome. *E.H. Yeon¹, E.J. Seo^{1,2}, M.J. Park¹, S.M. Park¹, Y.O Yoo¹, Y.H. Kim^{1,3}, J.K. Ko^{1,3}, H.W. Yoo^{1,3}, I.S. Park^{1,3}.* 1) Genome Research Center, Asan Medical Center, Seoul, Korea; 2) Dept. of Clinical Pathology, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea; 3) Dept. of Pediatrics, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea.

The 22q11 deletion syndrome is the most common genomic disorder on 22q11 caused by chromosomal rearrangements. The main clinical findings include characteristic facial appearance, velopharyngeal insufficiency, conotruncal heart defects, thymic hypoplasia or aplasia, and hypocalcemia. More than 90% of patients with 22q11 deletion have a similar 3-Mb hemizygous deletion, and 7 % of deleted patients have a nested distal breakpoint resulting in a smaller 1.5-Mb deletion. With the deletion of large genomic region, altered dosage of several contiguous genes is responsible for their etiology. To define the deletion boundaries and evaluate if the size of the deletion is correlated with the degree of severity of the phenotypes, we examined the genotype of the deletion and clinical features from 46 patients with the 22q11 deletion syndrome. Copy number to detect haploinsufficiency was analyzed in nine genes which are located within or in boundaries of 22q11 deleted region. These were CECR6, TUBA8, DGCR6, TUPLE, TBX1, COMT, MAPK1, VPREB1 and BCR. By using TaqMan probes specific to each genes, copy number of each gene was quantitated with a real-time PCR assay. The deleted maps in all of the patients were defined and compared with their clinical features. Twenty-five percents of patients had a smaller deletion spanning from TUBA8 to COMT. And we were unable to find any correlations between the size of the deletion and the phenotypic severity.

Identification of a gene as a candidate for involvement in the aetiology of premature ovarian failure. *S. Burnham*¹, *M. Waters*¹, *J. Wood*², *D. Vetrie*³, *E.M. Kendall*¹. 1) School of Biology, University of Leeds, Leeds, England; 2) Guys Hospital, Sts Thomas Street, London, England; 3) The Wellcome Trust Sanger Centre, Hinxton, Cambridge, England.

Premature ovarian failure affects approximately 1% of the female population. Several different types of chromosomal rearrangements have been described in patients with premature ovarian failure, these include deletions, X:Y translocations and X:autosome translocations. In 1998, Bione et al published details of a gene disrupted by the breakpoint of a X:autosome translocation in which the patient suffered from premature ovarian failure. The gene involved being the human homologue of the *Drosophila* diaphanous gene, mutations of the diaphanous gene are responsible for sterility in the fruit flies. Other genes have since been identified as candidates for involvement in the aetiology of premature ovarian failure by the virtue of their being located at translocation breakpoints of other patients, these include XPNPEP2 and FOXL2. We have been studying the location of the breakpoints of two further patients with balanced X:autosome translocations, the breakpoints being located in Xq21.3 and Xq22.3. We have narrowed down the positions of the breakpoints by using a series of methods including restriction mapping, the production of sub-clones, vectorette-PCR and Southern hybridisations. Sequence produced in this way has been compared with the public databases to identify transcripts from within the regions. The searches of the databases identified a gene which is disrupted by the translocation breakpoint of one of the patients we have been studying. We have performed RT-PCR using ovary RNA and have shown that this gene is expressed in the ovaries. We propose that this is another candidate gene for involvement in premature ovarian failure.

Human chromosome 21 gene expression atlas in the mouse. *V. Marigo*¹, *A. Reymond*², *M.B. Yaylaoglu*³, *A. Leoni*¹, *C. Ucla*², *N. Scamuffa*², *C. Caccioppoli*¹, *E.T. Dermitzakis*², *R. Lyle*², *S. Banfi*¹, *G. Eichele*³, *A. Ballabio*¹, *S.E. Antonarakis*². 1) TIGEM, Naples, Italy; 2) Division of Medical Genetics, Geneva University Medical School, Geneva, Switzerland; 3) Max Planck Institute of Experimental Endocrinology, Hannover, Germany.

The study of gene expression patterns is of crucial importance to the understanding of gene function. We set out to systematically analyze the expression patterns of genes from an entire chromosome. We chose human chromosome 21 (HC21) because its entire finished nucleotide sequence was available. Furthermore trisomy 21 (Down syndrome, DS) is the most common genetic cause of mental retardation. This chromosome-based approach has advantages over the study of an arbitrary set of genes, as it provides information on the consequences of aneuploidy, and may allow the identification of clusters of co-regulated genes. All identifiable murine orthologs of human chromosome 21 genes (161 out of 178 confirmed human genes) were analyzed by RNA in situ hybridization on whole-mounts (E9.5, E10.5) and tissue sections (E14.5), and by RT-PCR on adult tissues. These stages correspond to mid and late embryonic and fetal human periods, when the major organs and body regions are organized. Patterned expression was observed in several tissues including those affected in trisomy 21 phenotypes (i.e. CNS, heart, gastrointestinal tract, and limbs). Furthermore, statistical analysis suggests the presence of clusters of genes with significant patterns of either coexpression or lack of expression in specific tissues. The entire data set was incorporated in a web site that will be made available to the scientific community. This high resolution expression "atlas" of an entire human chromosome is a new level of gene annotation, which is likely to advance our knowledge on gene function and regulation and to the understanding of human aneuploidies, such as DS.

Characterizing the D21Z1 alphoid junction regions in the centromere of human chromosome 21. *M.R. Bozovsky¹, M.J. Roy¹, M. Flisak¹, J. So¹, M.R. Cummings², J.L. Doering¹*. 1) Dept. of Biology, Loyola University Chicago; 2) Dept. of Biological Sciences, University of Illinois at Chicago.

Centromeric and other heterochromatic regions are not included in the human genome sequence, although they comprise 10-15% of the genome. Sequences required for a fully functional centromere have not been fully defined, but alphoid repetitive sequences clearly play a role. We have constructed a detailed physical map of the centromere of chromosome 21 by direct pulsed field mapping. This map is linked to the q arm by the marker D21S190. The major centromeric alphoid cluster, D21Z1, is a 1.0 Mb long homogeneous array. A 0.3 Mb long satellite I cluster is located 0.25 Mb from the p arm end of D21Z1, and a TPTE gene is 0.3 Mb from the p arm end of the satellite I cluster. Characterizing the ends of the D21Z1 cluster may clarify evolutionary events in centromere formation and identify sequences potentially important for centromere function. The p and q arm ends of D21Z1 have distinctly different restriction maps, indicating the edges of the array do not have the similar organization predicted by the out-of-register recombination model for alphoid cluster evolution. BAC clone 21B49A22, the last clone in the q-arm sequence, spans an alphoid junction region containing non-repetitive DNA adjacent to 31.2kb of alphoid DNA. Analysis of this alphoid DNA shows no evidence of any of the regular repeating restriction sites or CENP-B boxes characteristic of higher-order alphoid repeats. Dot-plot analysis further indicates that this is monomeric alphoid DNA. The entire length of this region has a uniform 77-79% sequence similarity to D21Z1. The monomeric sequence forms an evolutionary clade distinct from D21Z1 and may contain chromosome specific sequences. We searched 1kb intervals of the monomeric alphoid DNA for regions with low similarity (<90%) to others in the current NCBI database, and identified several regions as putatively chromosome specific based on BLAST results. A chromosome 21 specific centromeric marker would be useful to definitively score parent of origin and stage of meiosis of a nondisjunction event or diagnose aneuploidy.

Evolutionary transposition of 4 unique genes mediated by flanking duplicons in the Prader-Willi/Angelman syndromes deletion region. *J-H. Chai*¹, *D.P. Locke*², *E.E. Eichler*², *R.D. Nicholls*¹. 1) Department of Psychiatry, University of Pennsylvania, Philadelphia, PA; 2) Department of Genetics, Case Western Reserve University, Cleveland, OH.

Prader-Willi and Angelman syndromes (PWS and AS) typically result from an ~ 4 Mb deletion of human chromosome 15q11-q13, with clustered breakpoints (BP) at either of two proximal (BP1, BP2) and one distal (BP3) site. *HERC2* or other duplicons at these BP regions are thought to mediate homologous recombination to generate 15q11-q13 deletions and duplications. We previously identified four non-imprinted genes (*GCP5*, *CYFIP1*, *NIPA1* and *NIPA2*) between BP1 and BP2, and have now characterized in detail the function and evolutionary origin of the BP1-BP2 region, particularly the *NIPA1/NIPA2* gene family. *NIPA1* and *NIPA2* each encode polypeptides with 9 transmembrane domains, suggesting function as receptors or transporters. Whereas *NIPA2* is constitutively expressed, *NIPA1* shows a central nervous system-specific expression pattern. By fusion to EGFP and microscopy, *NIPA1* is localized to vesicles within the cytoplasm and long processes of several cell lines (HeLa and mouse HN33 hippocampal neuronal cells), and similar studies with transgenic mice are under way. Phylogenetic analyses in 9 vertebrate, invertebrate and plant species indicates that *NIPA1* evolved ~ 450 million years ago by a genomic duplication of an ancestral *NIPA2* gene, whereas *NIPA2* is found in all multicellular organisms. Further evolutionary studies indicate that the 4-gene cassette between BP1 and BP2 in human is conserved but located adjacent to the single *Herc2* and flanking *p* (*OCA2*) genes in mouse and *Fugu*, genes that are adjacent to BP3 in human. These observations suggest a model in which duplications of the *HERC2* gene in primates first flanked the 4-gene cassette at the homologous position to 15q13 (BP3), with subsequent transposition of the entire cassette by a *HERC2*-duplicon mediated process to form the BP1-BP2 region 4 Mb proximal of the ancestral location. Duplicons appear therefore to mediate genomic fluidity in both disease and evolutionary processes.

Chromosomal evolution of human chromosomal region 11p14-15.5 and chromosome 12. *D. Prawitt, T. Enklaar, B. Keller, L. Brixel, C. Spangenberg, B. Zabel.* Molecular Genetics Lab, Children's Hospital, Univ. of Mainz, Mainz, Germany.

In an effort to localize all genes of the nucleosome assembly protein (hNRP) family, we isolated a PAC-clone specific for hNRP. FISH mapping experiments placed the gene into chromosomal region 12q21, providing further evidence for structural similarities between subregions on chromosome 11 and 12. Well known is the fact that the genes LDHA, HRAS1 and IGF2 on 11p14-15.5 have corresponding paralogous genes (LDHB, KRAS2 and IGF1) on chromosome 12 and because of their high genomic similarity, a common evolutionary descent of the two chromosomes was presumed (Walter et al., 1991). To analyse this situation in more detail we generated a list of paralogous genes on human chromosome 11p and on chromosome 12 enabling us to explain the genomic data as result of an evolutionary early duplication of a large genomic area, which then was further modified (under reduced selection pressure) by additional steps which might have included inversions or translocations. This model also explains the arrangement of the 11p gene group on two distinct chromosome 12 areas and the localization of corresponding orthologous murine genes on at least two chromosomes (6, 10). To our estimate the regions of chromosomes 11/12 paralogy seems to be limited to 11p14-15.5 on chromosome 11, since we could not determine genes located more centromeric on 11p characterized by gene family members on chromosome 12. An interesting consequence of a duplication event is the redundancy of gene products. According to Sidow (1996) two mechanisms could regulate a resulting increase of gene products: an alteration in the sequence of the duplicated gene leading to a different function of the produced protein, or a modification of a cis-regulating region that results in an altered gene expression. Cis-regulating genomic domains defining expression specificity of genes could be binding sites for transcription factors or modifiers. In this regard it is of interest that 11p15 is subject to epigenetic regulation, whereas this is not reported for the paralogous regions on chromosome 12.

Construction of Large Insert BAC Libraries of Gibbon and Spider Monkey. *R. Indugula¹, Y. Qian¹, L. Jin¹, B. Su^{1,2}*. 1) Center for Genome Information, Department of Environmental Health, University of Cincinnati, USA; 2) Key Laboratory of Cellular and Molecular Evolution, Kunming Institute of Zoology, Kunming, China.

Large insert genomic DNA library is a critical resource for genome-wide genetic dissection of target species. We constructed two high quality bacterial artificial chromosome (BAC) libraries of nonhuman primate species, the siamang (*Hylobates syndactylus*) and the black-handed spider monkey (*Ateles geoffroyi*). The clone vector, pBACe3.6 was used. A total of 240,000 and 200,000 BAC clones were generated respectively for the siamang and spider monkey libraries, respectively. The average insert size of the BAC clones was estimated to be about 135kb for the siamang library and 186kb for the spider monkey library. The small inserts (50-100kb) are less than 3% for both of the libraries constructed. Assuming a similar genome size with humans, the two libraries have about eleven times coverage of their genomes. Therefore, these two BAC libraries would serve as valuable resources in comparative genomic study and large-scale genome sequencing of non-human primate species. They will be made available for distribution upon request.

Program Nr: 1314 from 2002 ASHG Annual Meeting

Isolation of the full-length cDNAs for "predicted genes" and "novel genes" on human chromosome 21. *J. Kudoh, K. Shibuya, J. Wang, I. Obayashi, T. Sasaki, A. Shimizu, S. Asakawa, S. Minoshima, N. Shimizu.* Department of Molecular Biology, Keio University School of Medicine, Tokyo, Japan.

The chromosome 21 mapping and sequencing consortium including us finished 33.83-Mb genomic sequencing of chromosome 21 and identified 225 genes including 98 predicted genes (Nature, 405:311-319, 2000). We are now performing full-length cDNA isolation of "predicted genes" which were identified by sequence similarities to known genes and/or spliced EST matches and/or exon prediction programs. Putative exon sequences were used to amplify cDNA fragments by PCR using cDNAs from various human tissues. We identified nearly full-length cDNAs for predicted genes including DSCR6 (Down syndrome critical region gene 6), ANKRD3 (ankyrin repeat domain 3), ZNF295 (zinc finger protein 295), C21orf25 (chromosome 21 open reading frame 25), ZNF298, UMODL1 (uromodulin-like 1), TMPRSS3 (transmembrane protease, serine 3), TSGA2 (testis specific gene 2), SNF1LK (SNF1-like kinase), WDR9 (WD repeat domain 9), PRED44/C21orf11, and C21orf30, and two clusters of keratin-associated protein (KAP) genes on 21q22.11 and 21q22.3 which consist of at least 46 KAP genes (See abstract by Shibuya et al., this meeting). Many genes have various transcripts produced by alternative splicing and/or alternative use of transcription start sites. The detailed transcript map and sequence information are available through our Web site (<http://www.dmb.med.keio.ac.jp>). These information and resources will be useful for identification of disease related genes, especially those responsible for a particular phenotype of Down syndrome or bipolar affective disorder, and structural and functional analysis of each of these genes.

Towards a comprehensive understanding of LINE-1 evolution in hominoids. *L. Mathews, S. Chi, N. Greenberg, M. Ulz, I. Ovchinnikov, G. Swergold.* Department of Medicine, Columbia University, New York, NY.

Even though substantial physical and behavioral differences distinguish humans and the other great apes, many studies indicate that these species are very similar at the nucleotide sequence level. This raises the possibility that other types of genetic changes may account for some of the phenotypic differences between them. For example, the activities of retrotransposable elements may play important roles in species and genomic evolution. To investigate the role of LINE-1 (L1) amplification in the genomic evolution of the great apes we have created LOAF (LINE One And Flanking DNA) libraries of full-length, recent L1 insertions from their genomic DNA. Comparison with data from the human genome sequence indicates that the LOAF libraries contain random samplings of L1 insertions that amplified during recent hominoid evolution as well as a reduced sampling of older hominoid insertions. By determining the species distribution of many L1 insertions we have assembled the first description of the evolutionary ages of the L1PA# subfamilies. These data indicate that the different subfamilies have been amplifying for varying lengths of time. L1s that inserted during each of the major branches of hominoid evolution have been isolated and sequenced. These studies have yielded the first molecular phylogeny of hominoid L1s. Our data indicate that LOAF libraries are a potent method for studying L1 evolution and for developing powerful molecular characters for phylogenetic studies. Surprisingly, we have measured substantial differences in the rates of L1 amplification in humans, bonobos, and common chimpanzees. Because the activities of L1 insertions may have large effects on genome structure and function, we suggest that differences in L1 amplification rates between humans and other great apes reflect important differences in their evolutionary history. L1 amplification may have played an important role in the phenotypic divergence of humans and the great apes.

Haplotype analysis of the HLA-DR subregion belonging to the DR53 group. *H. Hohjoh*¹, *J. Ohashi*¹, *M. Takasu*¹, *T. Ishida*², *K. Tokunaga*¹. 1) Human Gen/Grad School Medicine, Univ Tokyo, Tokyo, Japan; 2) Biol Sci/Grad School Science, Univ Tokyo, Tokyo, Japan.

Phylogenetic trees for DRB1 alleles constructed by the neighbor-joining (NJ) and UPGMA methods using nucleotide sequences of the alleles suggest that DRB1*0701 (DR7 haplotype) may have diverged from other DRB1 allelic lineages before the separation of human and chimpanzee because of a large number of nucleotide changes in DRB1*0701 compared with any of the other DRB1 alleles. In our previous study, we found a processed pseudogene encoding the protein kinase, interferon-inducible double-stranded RNA-dependent activator (PRKRA), in the telomeric region of the HLA-DRB7 pseudogene. Interestingly, the PRKRA pseudogene appears to be specifically present in the DR4, 7, and 9 haplotypes belonging to the DR53 group, suggesting that the PRKRA pseudogene may have been generated in the common-ancestral genome of the DR53 group. Based on nucleotide changes in the PRKRA pseudogene, the divergence times of the DR4, 7, and 9 haplotypes were estimated to be 0.38~0.30 Myr ago. Thus, there is a significant difference between the phylogenetic trees and our observations. To examine this issue, we investigated haplotypes with the PRKRA pseudogene and the centromeric flanking region of DRB1 and also examined chimpanzee genome carrying Patr-DRB1*0701. Results indicate that the DR53-common ancestral haplotype carrying the PRKRA pseudogene may have been present before the separation of human and chimpanzee, and lead to the possibility that there may be a difference in the mutation rate of DRB1 gene among DR4, 7, and 9 allelic lineages.

Comparative analysis of gene expression patterns in human and African great ape cell lines. *M.W. Karaman¹, L.G. Chemnick², M.L. Houck², D. Sudano³, D. Chawannakul¹, S. Nagpal¹, O.A. Ryder², J.G. Hacia¹.* 1) IGM, USC, Los Angeles, CA; 2) CRES, San Diego, CA; 3) NHGRI, NIH, Bethesda, MD.

Little is known about the variations in gene expression patterns in human and the African great ape (chimpanzee and gorilla) populations. Since all three species have high protein sequence identity (>98.4%), differential gene expression may play a significant role in their phenotypic differences. A clear understanding of the intra- and interspecies variations in gene expression patterns is needed to uncover changes that lead to distinct phenotypes in these species.

We have used oligonucleotide microarrays to quantify mRNA levels in 22 human, 11 chimpanzee, and 11 gorilla early passage fibroblast cell lines from donors of different ages. Over 12,500 genes in every cell line were individually assigned a numerical 'expression level' score. Expression data from all cell lines from a given species were electronically pooled and used to generate average 'human', 'chimpanzee', and 'gorilla' expression level scores for all 12,500 genes. Approximately 40% of genes analyzed were expressed at appreciable levels in every cell line. Human and African great ape gene expression scores were compared and statistical measurements of intra- and interspecies variations in gene expression in relation to donor age and gender were calculated.

We identified genes expressed at significantly different (>3 fold) levels among the human and the African great ape cell lines. Greater than 70 genes were over-expressed in human relative to African great ape fibroblasts. Conversely, there were 16 genes that were over-expressed in African great ape relative to human fibroblasts. Several of the differentially expressed genes between human and African great ape fibroblasts were involved in oxidative stress responses and hereditary neurological disorders. Hierarchical clustering analysis was used to compare the relatedness of the gene expression profiles. Human, chimpanzee and gorilla gene expression profiles all clustered according to the species of origin. These experiments could shed light on fundamental biochemical differences among human and non-human primates.

Nine-bp deletion polymorphism of mitochondrial DNA in Korean population. *M.M. Oh, S. Han, Y. Jung, J. Oh.*
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We investigated the 9-bp deletion motif of the mitochondrial DNA (mtDNA) in Korean population. The 9-bp deletion motif is caused by the loss of one unit of the tandem repeat sequences in the tRNA^{Lys}/cytochrome oxidase 2 (*CO II*) intergenic spacer in human mtDNA. By the report, the coalescent time of the 9-bp deletion haplotypes has been estimated about 50,000-70,000 years before present (YBP), suggesting multiple independent origins from Africa, Europe, and Asia. In East Asia, the frequencies of the 9-bp deletion haplotypes were broadly different among various populations showing geographic cline. In this study, we analysed the 9-bp deletion motif using polymerase chain reaction, polyacrylamide gel electrophoresis, and DNA sequencing. Korean population showed polymorphic patterns in this mtDNA region as previous reports and the frequency was 17.9% (34/190). The hominid fossils identified as modern human, and the Paleolithic archeology records found in Korean peninsula, were estimated from 40,000 to 100,000 YBP. Consequently, the existence of 9-bp deletion leads an opinion that some maternal lineages of the ancient ancestors probably immigrated via ocean or continent after the first ancestors' settlement. We suggest that this study will give a good information to explain the history for the prehistorical migration pattern and tracing population affinities of Korean population.

Characterizing pericentromeric duplications: An expanded analysis of 2p11. *J.E. Horvath¹, M. Rocchi², S. Schwartz¹, E.E. Eichler¹*. 1) Dept Genetics, Case Western Reserve Univ, Cleveland, OH; 2) Istituto di Genetica, Universita di Bari, Bari, Italy.

Recent work in pericentromeric and subtelomeric regions of the human genome indicates that these regions are mosaics of paralogous segments. In an effort to better understand the evolutionary origin of the genomic structure in these regions we have focused our efforts on characterizing the pericentromeric region of chromosome 2 in detail. We have developed a ~650kb BAC contig from 2p11 abutting alpha satellite DNA and flanking 7 partial gene duplications and 3 pericentromeric interspersed repeats (PIR). The duplicated genic segments arose from 1p13, 2p12, 4q24, 7, 11p15, 22q11, and Xq28 and show 94-97% sequence identity to the ancestral loci. Based on PCR and FISH analyses, there are 5-8 copies of each duplicated genic segment distributed across multiple chromosomes. In contrast, there are more than 65 copies of PIR4 distributed among half of all human chromosomes suggesting multiple copies exist on each chromosome. Our analysis also indicates that larger blocks of sequence are shared between a subset of pericentromeric regions, such as 2, 10, 16 and 22, suggesting that these regions have undergone vast restructuring events in recent evolutionary time. PCR and comparative FISH data indicate that both the genic duplicons and PIRs are multicity in chimpanzee and gorilla but are single copy in orangutan. Phylogenetic analysis suggests that five of the seven genic duplicons were transposed to an ancestral pericentromeric region around the same time (5-8 mya). The duplicated PIR4 element, however, arose earlier in evolution (>12 mya) and may have pre-existed many genic duplicons. Combined, the data indicate that this region of 2p11 has accepted duplicated material at a punctuated time in evolution. Consequently, there is significant variation between closely related species. Based on our data we propose a model to explain the organization of human pericentromeric DNA and more importantly, a mechanism of rapid evolutionary turnover involving the transposition and juxtaposition of genic and repetitive segments.

Nature and Pattern of Primate Genomic Variation. *G. Liu*¹, *NISC Comparative Sequencing Program*², *E.D. Green*², *E.E. Eichler*¹. 1) Department of Genetics, Center for Human Genetics and Center for Computational Genomics, Case Western Reserve University School of Medicine and University Hospitals of Cleveland, Cleveland, OH; 2) NIH Intramural Sequencing Center, Gaithersburg, MD.

We performed a detailed analysis of both single nucleotide and large-scale insertion/deletion (indel) events based on large-scale comparative analysis of 15 Mb of sequence from baboon, chimpanzee and human. Optimal global alignments were constructed from 40 orthologous loci and examined for the pattern, frequency and nature of mutational events over an estimated 25 million years of human evolution. Partitioning the genome sequence into different classes reveals significant differences between exonic, non-coding unique DNA and repetitive DNA with higher rates being observed for Alu/L1 DNA when compared to non-coding DNA. The overall single-base pair mutation rate was calculated as $\sim 1.15 \times 10^{-9}$ events per site per year which is significantly lower than the previously reported values. Mutation rates were very comparable between two sets of sequence alignments (human vs. chimpanzee and human vs. baboon) supporting a constant molecular clock hypothesis while arguing against the hominoid rate-slowdown hypothesis. We investigated the potential origins of large-scale indel events (>100 bp). Alu and L1 were found to mediate the majority of recent retrotranspositions showing strong species bias. Based on 5 Mb of aligned orthologous sequence, we identified 153 Alu events in baboon while only 96 in human, suggesting a slowdown of Alu activity within the hominoid lineage. Further, for human-chimpanzee sequence alignments, a two-fold increase in Alu (22:11) and L1 (9:5) insertions was detected in human when compared chimpanzee. Finally, an analysis of de novo deletion events found that more than two-thirds of them shared a SINE or LINE element precisely at the deletion boundaries, suggesting homologous recombination between common repeat elements as a mechanism for their origin. The data provide the first global insight into the mutational forces that have shaped the human genome and provide a valuable baseline for the identification of regions that deviate from the neutral expectation of genomic mutation.

Evolutionary Genomics of Transcription Factor Brain-2 in Mammals. *L. Wang*¹, *F. Mizuno*², *S. Ueda*². 1) Medical & Animal Gen, Inst Gen, Chinese Academy Sci, Beijing, China; 2) Department of Biological Sciences, Graduate School of Science, University of Tokyo, Tokyo, Japan.

We have previously found a unique evolutionary feature of transcription factors; (1) transactivation domain of the transcription factors had amino acid structure unique to each class of vertebrates, and (2) those unique features were represented by the presence of homopolymeric amino acid repeats (sequences without interruptions in the run of a single amino acid residue) including alanine, glycine, and proline. Presence/absence of these amino acid repeats, which are encoded by triplet codons GCN, GGN, or CCN, are well concordant with GC contents of their genes. Transcription factors having higher GC contents show higher contents of alanine, glycine, and proline residues, while transcription factors having lower GC contents show lower contents of these amino acid residues. This correlation is observed even among various types of transcription factors from vertebrates and invertebrates, regardless of functional and structural constraints inherent to each transcription factor. Here we newly obtained three hominoid (chimpanzee, gorilla and orangutan) Brain-2 genes and compared their nucleotide and amino acid sequences with those of the vertebrate orthologues including the human and mouse ones. Brain-2 is one of the transcription factor class III POU, which plays an important role in nervous system. All of the mammalian Brain-2 had alanine-, glycine-, proline-, and glutamine-repeats, which are missing in the non-mammalian orthologues. Moreover, these characteristic homopolymeric amino acid repeats are highly conserved in both position and repeat number among mammals.

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Mutation, methylation, mismatch, and Man: exploring the human mutome. *A. Platt.* Organismic and Evolutionary Biology, Harvard University, Cambridge, MA.

Recent large-scale SNP-mapping techniques have given us unprecedented tools in examining evolutionarily recent mutations in humans. By describing the distribution of 8.2×10^5 SNPs described by The SNP Consortium in terms of short (six flanking nucleotides), medium (sequence words within 50 nucleotides), and long (GC content of 100 nucleotide neighborhood) distance sequence contexts I show dramatic (260 fold) variability in levels of polymorphism at different classes of sites. This genomic approach offers insight into the mutational propensities of human DNA replication and includes implications for an extended role of CpGs beyond deamination-induced hypermutability.

Biases against Microsatellites in the Human Genome: implications for genomic instability. *J.E. Stenger, Y. Liu.* Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC.

Whether microsatellites are advantageous or pose a threat to DNA stability is dependent on their length. We, therefore, undertook a study to find all microsatellites with repeat units under seven nucleotides (homonucleotide runs through hexanucleotide runs) in the UniGene set to identify biases against length. First we looked at homonucleotide runs since their hypermutability in mismatch repair and DNA polymerase proofreading yeast mutants was experimentally demonstrated (Tran et al., 1997). Only the mismatch repair system prevents frameshift mutations in runs greater than or equal to eight consecutive nucleotides. So we decided to determine if there was a bias in the genome of against such tracts. We looked independently at the four possible types of homo-nucleotides (A, C, G and T runs) greater than or equal to runs of six homo-nucleotides. Of these tracks of length six, the A tracks were most common: 6,296 occurrences of six As versus 4444 for C, 2711 for G and 2574 for T. The number of occurrences dropped off dramatically between length 8 runs and length 9 runs. The number of occurrences for A, C, G and T of length 9 were 124, 10, 6, and 41 respectively. Thus, the experimental data was corroborated by a bias against sequences that are potentially unstable and we can see this reflected in the genome following computational analysis. We then reasoned that for more complex microsatellite repeats, observing such drop-offs in the number of occurrences, as the number of iterations of the repeat increases would also reflect instability. So we did an analysis of singly interrupted homo-nucleotides (as these are predicted to have a similar effect on DNA structure as homo-nucleotide repeats, if the sequence is long enough), and di- through hexa-nucleotide repeats. We found biases against interrupted nucleotides with an overall length of greater than 11. In addition we found that di-nucleotide repeat numbers dropped off significantly after 5 iterations (overall length 10), tri-nucleotides after 8 iterations, tetra-nucleotides after 3 iterations, penta-nucleotides after 2 iterations, and hexanucleotides after three iterations.

Missing in action: search for unsequenced human genome regions. *J.R. Korenberg¹, X-N. Chen¹, P. Bhattacharyya¹, SY. Zhao², H. Shizuya³, M. Simon³.* 1) Med Genet, Cedars-Sinai Med Ctr/UCLA, Los Angeles, CA; 2) TIGR, Rockville, MD; 3) Caltech, Pasadena, CA.

The human genome reflects a rich history of duplication events that mark regions of contemporary variability and instability. In order to provide an alternative approach to identifying unstable and duplicated regions that does not depend on internal analyses of the draft sequences, we analyzed BAC end sequence (BES) from a subset of 6,000 BAC clones identified at random (BAC Libraries A & B) that generate multiple signals by FISH on human metaphase chromosomes. A total of 580 BES representing 397 multiple-site BACs were analyzed against the UCSC draft sequence (Aug 01 & Apr 02) and Celera draft (2001). The results of the UCSC Aug 01 searched in masked format against unmasked BES revealed that from the 580 BES (179 BACs with both BES), 293 had no hits, 137 were low hits (90-97% homology) and 150 were high hits (98%). However, 8 of 13 BACs that hit the same chromosome were at the correct spacing (80-300Kb). The results of the UCSC Apr 02 searched in unmasked format against unmasked BES showed from a subset of 98 BES (49 BACs with both ends), 32 BACs had high hits, of which 13 hit the same chromosome, although only 10 out of 32 had the correct spacing. When searched masked, one out of 32 remained a high hit with both ends matching to the same chromosome albeit with incorrect spacing. These results indicate that the progress of the draft sequencing had little effect on the set of BES with no match. The Celera database search revealed that out of 580 BES, 5 had no hits, 575 had hits, of which, 132 were low hits and 443 were high hits. However 168 of 443 hit the same chromosome, of which 54 had the correct spacing. In addition, 61 BACs hit orphan contigs, with 27 that had both BES hit only orphan contigs. Analyses of both draft sequence sources suggest that at least 65% (UCSC) and as much as 90% (Celera) of multisite BACs identified above were not included or misassembled in the current draft sequence. Therefore, these missing sequences identify gaps in the draft sequences and provide links for defining regions of genomic instability involved in germline and somatic disease.

Human Genes of Ancient Origin. *G.A. Bruns*^{1,2}, *R.E. Eisenman*¹. 1) Genetics Division, Children's Hosp, Boston, MA; 2) Dept of Pediatrics, Harvard Medical School, Boston, MA.

Various categories of human genes of unknown function may be of interest to identify. Examples include those with predominant expression in one organ system; those with expression at very early stages of development or fleeting expression in a developmental process; or those with significant expression only in primates. We have focused on finding novel human genes that have extensive homology in *D.melanogaster* and *C.elegans* but do not recognize known proteins, protein families or motifs in current databases. These encode new families of proteins of ancient origin. As extensive cross-phylum AA sequence conservation is frequently a hallmark of proteins involved in fundamental cellular processes, function analysis of some of the proteins may elucidate priorly unknown pathways. Mutation at loci encoding ancient sequences also often underlie human inherited disorders.

More than 40 genes of this class were recognized by database search. Human unique Unigene sequences were BLAST analyzed to find those with significant homology (E values, e^{-005} to e^{-067}) in the *D.melanogaster* EST database. Any initial match to a homolog of known function or a defined motif in the Unigene or nr db was excluded. For each candidate the human protein was sought from the nr database which often yielded the *D.melanogaster* relative. The fly homolog was also obtainable from FlyBase. For all but 4 genes, a *C.elegans* homolog was retrieved from the nr or Wormpep databases. In some cases Wormpep or FlyBase gave identification of a gene not available from the nr annotation. Such loci were excluded. This indicates the importance of using multiple databases when seeking novel genes. All candidate genes were analyzed with pfam. The human proteins were aligned to the fly and worm homologs using the NCBI BLAST 2 Sequences algorithm. The E values ranged from e^{-05} to e^{-135} with the majority at least e^{-30} . EST representation for the human genes was retrieved from the Unigene db. The gene identifiers, homology relationships of the proteins plus RNA data will be presented and posted at <http://tnt.tch.harvard.edu/bruns/>.

Finding of New Genes from Proximal One-Third (cen-q11.2) of Human Chromosome 22. *T. Sasaki, A. Shiohama, K. Hosono, H. Yang, S. Asakawa, J. kudoh, S. Minoshima, N. Shimizu.* Dept Molecular Biol, Sch Med, Keio Univ, Tokyo, Japan.

We previously reported the first sequence analysis of human chromosome 22q (33.4 Mb) and identified 545 genes including 298 related and predicted genes (*Nature* **402**: 489-495, 1999). Since then, we have continued more extensive analysis by manual evaluation of the computer predictions and experimental isolation of cDNAs aiming at a complete transcript map. To date, we finished analyzing the proximal one-third of 22q (10 Mb), which includes the Cat Eye Syndrome (CES) region, the DiGeorge Syndrome (DGS) region and the IGL-IGLL region. We identified 15 new genes from these regions. Here, we report the initial characterization of some of these new genes.

DGCRK7 is located between T10 and HTF9c genes in the DGS region. Northern blot analysis revealed that DGCRK7 is transcribed in three forms, and expressed ubiquitously in various organs/tissues of both human and mouse origins. We also isolated a cDNA of mouse homolog Dgcrk7 that has 95.3% amino acid identities with human DGCRK7. The protein domain analysis revealed one WW motif and two double stranded RNA binding motifs. This gene may be associated with some clinical phenotypes of DGS.

PIWIL3 is a novel member of the PIWI/eIF2C family, and is located between GGT.2 and TOP1P2 in the IGL-IGLL region. A cloned cDNA was 3,504 bp in length including ORF of 2,652 bp. The PIWIL3 protein has PAZ and Piwi motifs as a common architecture of the PIWI/eIF2C family. PIWIL3 is expressed mainly in the testis. It is noted that the *Drosophila piwi* is associated with self-renewing of germline stem cell.

LUK1 locates between PPIL2 and PRKM1 in the IGL-IGLL region. LUK1 homolog is found in many eukaryotes including animal, plant and fungus, and hence this gene is considered to have a fundamental function. Through searching the human genome database, at least 4 more genes with high homology were found. The full-length cDNA cloning and functional analysis of all the remaining genes are in progress.

Functional autosomal copies of the X-linked human ribosomal protein genes. *N. Kenmochi*¹, *T. Uechi*², *T. Tanaka*².
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The mammalian ribosome is a massive structure composed of 4 RNA species and 79 different proteins (RPs). Unlike the RNA constituent, each protein is typically encoded by a single intron-containing gene. However, there are dozens of intronless copies of the active gene by retrotransposition at sites dispersed throughout the genome, though the majority of these copies are inactive. In this study, we have identified functional autosomal copies of the X-linked human RP genes and have designated these genes as *RPL10L* (ribosomal protein L10-like gene), *RPL36AL*, and *RPL39L* after the original ones. Because these genes have no introns in the coding regions, they are likely retrotransposed from the X-linked genes. The identities between the retrotransposed and original genes are 89%-95% in the nucleotide sequences and 92%-99% in the amino acid sequences, respectively. The Northern blot and PCR analyses revealed that *RPL10L* and *RPL39L* were expressed only in testis, whereas *RPL36AL* was expressed ubiquitously. Although the role of the second genes remains unclear, they might have evolved to compensate the dosage of the X-linked RP genes.

From Genomic to Representational Mismatch Scanning. *H.G. Thormar^{1, 3}, G.H. Gunnarsson^{1, 2}, B. Gumundsson², S.M. Gustafsdottir¹, S.M. Weissmann⁴, J.J. Jonsson^{1, 3, 4}.* 1) Dept. of Biochem. and Mol. Biol., Univ. of Iceland Medical School, Reykjavik, Iceland; 2) BioCule Inc., Reykjavik, Iceland; 3) Dept. of Clin. Biochem., Landspítali-University Hospital, Reykjavik, Iceland; 4) Dept. of Genetics, Yale School of Medicine, New Haven, Connecticut.

Genomic mismatch scanning (GMS) to identify identical-by-descent (IBD) sequences shared by affected relatives would have many potential applications. The human genome is too complex for efficient reannealing following denaturation, a requirement for GMS. We have developed a method Representational Mismatch Scanning (RMS) to generate and process representations of the human genome for various genomic applications including mismatch scanning. Adenine-rich sequences flanking 3' ends of Alu repeats (AluR3'Fl), many of which are highly polymorphic, were amplified from genomic DNA. Estimation of efficiency of reannealing and isolation of polymorphic, reannealed AluR3'Fl was done using two-dimensional conformation-dependent electrophoresis (2D-CDE) developed by our lab. Three rounds of subtractive hybridization were performed to identify non-fixed Alu repeats (polymorphic as to presence or absence) and recovered material cloned and analysed. Sequence analysis and Blast search on 27 clones from AluR3'Fl amplification confirmed that these were all AluR3'Fl and placed 21 of them on 11 chromosomes. In contrast to whole genome preparations AluR3'Fl representations could reanneal efficiently. Using subtractive hybridization we were able to clone AluR3'Fl adjacent to non-fixed Alu repeats further confirming favorable reannealing characteristics and demonstrating use of this technology in genome biology. Seven out of 14 (50%) reannealed AluR3'Fl sequences isolated after 2D-CDE were heteroduplexes due to polymorphism. The AluR3'Fl representations have several characteristics associated with an ideal human genome representation for mismatch scanning. They can be used in subtractive hybridization to isolate polymorphic non-fixed Alu repeats and in junction with 2D-CDE to isolate polymorphic AluR3'Fl. Polymorphic AluR3'Fl appear to have characteristics favorable for comparative genome hybridization to directly identify IBD sequences.

Characterisation and modification of human alphoid BACs and PACs for chromosome formation in mammalian cells. *T. Kaname*^{1, 4}, *K. Osoegawa*², *P.J. de Jong*², *P.A. Ioannou*³, *K. Naritomi*⁴, *C. Huxley*¹. 1) MAC group, MRC Clinical Sciences Centre, Imperial College, London W12 0NN, UK; 2) Childrens Hospital Oakland Research Institute, Oakland, CA-94609; 3) The Murdoch Institute for Research into Birth Defects, Royal Childrens Hospital, Melbourne, Australia; 4) Department of Medical Genetics, University of the Ryukyus School of Medicine, Nishihara, Okinawa, Japan.

Mammalian artificial chromosomes (MACs) are a potential tool for gene therapy, for transgenic animals, and for study of chromosome biology. Towards making reliable MAC vectors, we are investigating which alphoid DNA can frequently form a centromere *de novo* after transfection into cells.

We investigated the structure of 30 alphoid BACs and 20 alphoid PACs selected by screening human BAC and PAC genomic libraries with alphoid DNA. According to their sequences at both ends, FISH onto human metaphase spreads, and the pattern of digestion with several restriction enzymes, we have characterised BAC and PAC clones which have pure alphoid DNA from chromosomes 1, 7, 17, 18, 19, 21/13, and 22/14. In order to introduce and monitor these BACs and PACs in mammalian cells, we modified them by retrofitting with newly constructed plasmids using Cre-*loxP* recombination. The plasmids contain a *loxP* site for retrofitting, a spectinomycin resistant gene for selection in bacteria, a neomycin resistant gene expression cassette for selection in mammalian cells, and an EGFP expression cassette driven by the very strong CAG promoter. Retrofitting of a variety of BACs and PACs with these plasmids was frequent and accurate. Using the modified BACs and PACs, we could easily monitor the presence of the vector in HT1080 cells and mouse embryos. Then we investigated the potentiality of *de novo* MAC formation for the modified clones. All the vectors harbouring alphoid DNAs from chromosomes 17, 18, 21/13, 22/14 formed MACs with the frequency between 8.0×10^{-7} and 4.3×10^{-5} /lipofected HT1080 cells.

Trinucleotide repeats database from human chromosome 21 and 22. *H. Rhee*¹, *Y.-M. Lee*², *J.-S. Lee*^{1,2}. 1) Brain Korea 21 Project, Yonsei University College of Medicine, Seoul, Korea; 2) Department of Clinical Genetics, Yonsei University College of Medicine, Seoul, Korea.

Trinucleotide repeats (TNRs) are frequently found in the protein coding regions of human genome and the copy number polymorphisms of TNRs are associated with the various neurodegenerative disorders like Huntington's disease, spinocerebellar ataxia and spinobulbar muscular atrophy. These trinucleotide repeat expansion (TRE) diseases commonly show the genetic anticipation feature which refers to increased disease severity and earlier age of onset in successive generations. Because major psychoses like schizophrenia and bipolar disorder also show the genetic anticipation feature, the candidate causative genes for these diseases are actively investigated among the genes which have TNR copy number polymorphisms. In this study all the 64 types of TNRs in the genomic sequence draft data of human chromosome 21 and 22 are scanned *in silico* using locally developed computer program eTRF(Exact Tandem Repeats Finder). The results are integrated into the database to facilitate the identification of the various disease genes showing TRE and genetic anticipation.

Program Nr: 1331 from 2002 ASHG Annual Meeting

Sequence Validation of Cystic Fibrosis Mutations Detected with PCR/OLA Technology. *S.M. Koepf, L.Y. Nguyen, E. Schreiber.* Celera Diagnostics, Alameda, CA.

The cystic fibrosis transmembrane conductance regulator (CFTR) gene spans over 250 Kb with 27 exons and codes for a protein of 1480 amino acids. Mutations in this gene are responsible for causing Cystic Fibrosis (CF), the most common severe, autosomal recessive disease in caucasian populations. There have been over 1000 variants identified in this gene since it was first sequenced in 1989. Because heterozygosity of CF mutations can be quite common (1/25 in caucasians) and certain mutations have more severe consequences than others, there has been an increase in the demand for genetic testing of CF. In 2001, the American College of Medical Genetics (ACMG) set up guidelines for such testing and recommended 31 informative loci for screening. We have developed a simple, one tube assay, which follows the ACMG guidelines. This assay is based on a multiplex PCR followed by an oligo ligation assay (OLA). The OLA products are fluorescently labeled with one of 3 different dyes and separated and sized on an Applied Biosystems ABI Prism® 3100 Genetic Analyzer. Along with a review of this technology, fluorescent sequencing data are shown that validate the OLA results. Data are shown for several samples from Coriell's CF mutation panel, with special attention given to the 5/7/9 T polymorphisms located in intron 8 associated with splicing defects of exon 9.

Characterization of a duplicon unit, LCR7-20, in the human genome. *X.D. Liu, X. Li, M. Li, S.W. Scherer, Y.J. Acimovic, X. Estivill, L.C. Tsui.* Department of Genetics, Hospital for Sick Children , Toronto, Ontario, Canada.

DNA sequence duplication plays a pivotal role in genome evolution. Our previous study described the amplification of a genomic sequence containing exon 9 of the CFTR gene in human genome. We hereby report that this CFTR sequence is part of a large duplicated sequence unit, named LCR7-20 (low copy repeat 7-22). By constructing a human chromosome 7-specific cosmid contig and partially genomic sequencing in combination with existing human genome sequences, we assembled a 373 kb genomic sequence containing a prototype LCR7-20 sequence (LCR7-20.1) located on 7p11-12. Sequence search in the public databases revealed six additional copies of LCR7-20 with more than 90% sequence identity. Among them, two genomic contigs on chromosome 20 (LCR7-20.2) and 15 (LCR7-20.4) contain the complete LCR7-20 sequence and the others are single BAC/PAC clones containing the unfinished sequence fragments assigned to the chromosomes 6, 12, 15 and 20. Sequence alignment of seven LCR7-20 sequences suggested a unit length of LCR7-20 to be between 68.5 to 245 kb. Among them, three LCR7-20 sequences contain the CFTR exon-9 insertion. Detailed sequence analysis revealed that LCR7-20.1 is positioned by two 53 and 61 kb reverse repeats at both ends. The similar result was also observed on LCR7-20.2, which contains two highly identical 92-kb reverse repeats at its ends. Screening human BAC/PAC libraries, restriction mapping and sequencing the regions without short interspersed repetitive sequences, we found that there are more than 30 copies of LCR7-20 in the human genome and these LCR7-20 are roughly more than 120-kb long. FISH and comet-FISH analyses revealed that LCR7-20 units are located on different chromosomes, mainly in the centromeric and pericentromeric regions, as both single repeat and multiple tandem repeats. LCR7-20 therefore represents a duplicated unit that could undergo large complex sequence amplification during the human genome evolution. Our study also pointed a number of large sequence segments that may be mis-assembled. Further characterization of additional segmental duplication units will be important in correct assembly of the complete human genome.

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The Complete DNA Sequence of the Human Dystrophin Gene. *M. Maheshwari¹, S. Scherer¹, K. Worley¹, D. Wheeler¹, F. Yu¹, E. Sodergren¹, D. Muzny¹, J. Belmont¹, G. Weinstock¹, R. Gibbs¹, A. Coffey², G. Howell², D. Grafham², K. McLay², C. Bird², S. Whitehead², J. Ashurst², J. Rogers², M. Ross² and The DMD Gene Sequencing Collaboration**. 1) Dept Molec/Human Gen, Baylor College of Medicine, Human Genome Sequencing Center, Houston, TX; 2) Wellcome Trust Sanger Institute, Cambridge, UK.

Duchenne muscular dystrophy (DMD) is one of the most common and severe forms of muscular dystrophy and is caused by mutations in the dystrophin gene located at Xp21. Though the gene was cloned many years ago, its complete genomic structure has only recently been determined. It is one of the largest known genes; with a coding sequence of 14 kb spread over 2400 kb of genomic DNA. Much of the genomic sequence was included in the international collaborative effort that resulted in a draft Human sequence in June 2000. Subsequently a collaborative effort between the Baylor College of Medicine Human Genome Sequencing Center and the Wellcome Trust Sanger Institute has finished all BACs and PACs in a minimal tiling path across the whole gene. Analysis of the gene is underway, including precise definition of repeat content, gene prediction by different algorithms and cross species comparisons. In addition we are genotyping multiple markers to examine the conservation of linkage disequilibrium across the locus. These studies will show both the interesting architecture of this large gene and offer new reagents for DMD diagnosis.

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Genomic Sequencing and Analysis of the Human Chromosome 8q22-q24.1. *A. Shimizu, S. Asakawa, T. Sasaki, S. Yamazaki, S. Ishikawa, J. Kudoh, S. Minoshima, N. Shimizu.* Dept Molecular Biol, Keio Univ Sch Medicine, Kanagawa, Japan.

The human genome sequencing project is approaching to the proposal goal in Spring 2003 by the enormous efforts of international genome sequencing consortium. As a member, we have been involved in the sequencing and analysis of human chromosome 8q22-q24.1 This region contains several disease genes responsible for developing such as glaucoma, Cohen syndrome and myoclonic epilepsy and so on. The length of 8q22-q24.1 (SGC35251-D8S1804) region was estimated to be about 30 Mb which we completely covered with more than 400 Keio BAC clones. These BAC clones were processed for shotgun sequencing in combination with primer walking. To date, we finished sequencing of 140 BAC clones, covering 57% (~17.2 Mb) of this region. Together with the sequence data generated by other groups, 87% (~25.9 Mb) of this region has been covered with finished sequence and processed for exon prediction analysis and homology search with the database. We have so far found 18 novel genes (C8ORFK1, C8ORFK2, C8ORFK3, C8ORFK4 etc.) which have been confirmed by RT-PCR assay using the predicted exon sequences, RACE and Northern blot analysis. We also identified 96 known genes (PGCP, LC27, MATN2, RPL30, UK114, POP1, PRO1097, KRS1, GEM, CDH17, PABP, HTPHLP, P53R2, HYD, ODFPG, TIEG, DPYS, ST7, OXR1, INT6, ANGPT1, DC6, EBAG9, TRPS1, EXT1, EIF3S37 etc.) and 65 pseudogenes. The status of gene finding and mutations search will be presented.

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Reduction in feature size allows for accurate resequencing and SNP discovery for less than \$0.02/base. *N.A. Shah, Z. Zhang, J.A. Warrington.* Health Management Research, Affymetrix, Santa Clara, CA.

Oligonucleotide microarrays are a very powerful tool for gene expression monitoring. Here we describe another application for oligonucleotide microarrays, resequencing and SNP discovery. With the recent completion of the draft sequence of the human genome and the completion of sequencing projects for dozens of model organisms, this technology provides a rapid, economical and competitive method for identifying sequence variants. We have demonstrated that we are able to query 60 kb of sequence on a 1.28 x 1.28 cm microarray containing a total of ~340,000 unique probes. Ideally, genomic regions are amplified by long distance PCR, LPCR, and several fragments are pooled together at equimolar concentrations. Pooled amplicons are fragmented by DNASE 1 and end labeled using terminal transferase and biotinylated ddATP. The labeled target is hybridized to the microarrays overnight; arrays are washed, stained and scanned using an Affymetrix GeneChip system. Data is analyzed using GeneChip software. Using this system, excluding labor, the cost for 2x resequencing is less than \$0.02/base. In recent experiments, we are evaluating performance of a smaller feature size which increases the amount of sequence read per microarray and reduces the cost of labor and reagents per sample. Results of these experiments including validation data addressing accuracy and reproducibility will be reported.

Mapping the Methylation Status of CpG Islands located in upstream promoter regions and its relevance in Complex Genetic Disorders. *I. Yakub, R.A. Gibbs.* Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX.

Changes in DNA methylation profiles have been associated with a number of human diseases such as cancer and imprinting disorders like Beckwith-Wiedemann and Prader-Willi/Angelman syndromes. Variations in methylation status of CpG islands have also been linked to a number of mental disorders such as Rett Syndrome and X-linked alpha-thalassemia/mental retardation syndrome (ATR-X). Abnormal methylation patterns are known to alter levels of gene expression that may produce disease phenotypes. Although, analyzing the global methylation status of genomic DNA from patients is a good first step, it is not sufficient. A more detailed mapping of methylation patterns of individual CpG dinucleotides located in the promoter regions of candidate genes is essential if we are to fully understand the biological processes responsible for gene silencing in cancer and other genetic disorders as well as gene regulation of imprinted genes.

Methylation patterns are being mapped by scanning the CpG islands in the promoter regions of candidate genes from patient genomic DNA. The CpG islands are determined by the CpG plot software available on the European Bioinformatics Institute website. The assay involves the initial treatment of genomic DNA with sodium bisulfite, which chemically modifies all unmethylated but not methylated cytosine to uracil. Next, the CpG islands of interest are amplified with primers designed to amplify sodium bisulfite modified DNA only. These PCR amplified fragments are sequenced using the ABI Prism 3700 DNA Analyzer. Unmethylated Cytosines that have been modified to uracil will base pair with Adenine and therefore show up as As in the sequence while methylated cytosines will show up as Gs in the sequence. CpG islands from bisulfite modified patient DNA will be compared to sequence from bisulfite modified control genomic DNA to study the difference in methylation profiles and determine the functionality of the CpG islands. This method is being applied to study methylation profiles of CpG islands located in promoter regions of candidate genes in Schizophrenia and other complex genetic disorders.

High-throughput and cost-effective SNP typing method using single-molecule fluorescence detection system. *M. Bannai*^{1, 2}, *T. Akesaka*¹, *K. Hori*³, *K. Sato*³, *M. Kawashima*¹, *K. Tokunaga*¹. 1) Department of Human Genetics, University of Tokyo, Tokyo, Japan; 2) Genome Medical Business Division, Olympus Optical Co., Ltd., Tokyo, Japan; 3) NovusGene Inc., Tokyo, Japan.

Fluorescence correlation spectroscopy (FCS) is a powerful tool for characterizing fluorophores in solution at the single-molecule level by analyzing fluorescence fluctuations. The FCS method measures two important molecular parameters: average number and size of molecules in a tiny volume of solution (1 femtoliter). In combination with the PCR-sequence specific primer (PCR-SSP) method, we developed a high-throughput and accurate typing system of single-nucleotide variations. Sequence-specific primers separately labeled with different fluorescent dyes were used for dimorphic alleles and PCR-SSP reaction was performed competitively in the same solution. Since movements of DNA fragments in a solution depend on their sizes, primers (smaller molecules) move faster than PCR-amplified fragments (larger molecules) in the PCR product solution. After a narrow laser beam spots DNA fragments at a very small area in the solution, the signals of fluorescence-labeled molecules are detected by a highly sensitive spectrometer, then the numbers and sizes of both primers and amplified fragments are determined. A novel automated instrument for the FCS measurement allows almost-hands-free genotyping of hundreds of samples. For assay validation, one hundred different genomic samples that had been typed by PCR-single-strand conformation polymorphism (PCR-SSCP) were analyzed using this system. The genotypes determined by the PCR-SSP-FCS method were 100% concordance with the results determined by the PCR-SSCP method. More than 200 different SNPs have been tested so far, and more than 95 percent of them could be successfully distinguished. Even whole-genome-amplified samples could be typed, thus valuable samples with small amounts of genomic DNA are applicable to SNP typing. Accordingly, PCR-SSP-FCS is a useful method for reliable, cost-effective, and high-throughput typing of various SNPs.

Clinical application of a high resolution genomic microarray: detection of 6p25 deletions and duplications in patients with eye anomalies. *R. Ekong¹, S. Jeremiah¹, D. Judah², O. Lehmann³, F. Mirzayans⁴, M.A. Walter⁴, S. Bhattacharya³, T. Gant², S. Povey¹, J. Wolfe¹.* 1) The Galton Laboratory, Department of Biology, University College London, UK; 2) MRC Toxicology Unit, University of Leicester, Lancaster Road, Leicester, UK; 3) Dept. Molecular Genetics, Institute of Ophthalmology, Bath Street, London, UK; 4) Ocular Genetics Laboratory, University of Alberta, Edmonton, Canada.

Defining the extent of chromosomal anomalies by conventional methods is time consuming. Comparative genomic hybridisation has been used on arrays of BAC and PAC clones to detect copy number changes. We evaluated whether smaller clones (cosmids and cosmid-sized restriction digested (RD) fragments) provided a higher resolution using clinical samples from patients with duplications and deletions in 6p25.

We established a genomic array of BACs, PACs, cosmids and cosmid-sized RD fragments on aldehyde treated glass slides. These clones included the region of interest on chromosome 6p25, and other human chromosomes including X and Y. Patient and reference genomic DNAs were differentially labelled with fluorochromes and co-hybridised. After post-hybridisation washes, the slides were scanned and analysed.

Patient samples and unaffected controls were analysed blind and the extent of each chromosomal aberration was correctly identified in all cases using the PACs/BACs in the array. Results from cosmids and RD fragments within the region were consistent with those from the larger insert clones. These results have been confirmed independently by other methods.

This study demonstrates that cosmid-sized genomic clones can be successfully used in genomic microarrays and that they potentially provide a higher resolution than PACs and BACs. Their use should facilitate identification of cytogenetic breakpoints and assist in patient diagnosis.

2-D Conformation-Dependent Electrophoresis for analysis of complex DNA samples (2D-CDE). G.H.

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DNA fragments containing mispaired and modified bases, bulges, A-tracts, and cruciform structures have altered conformation. Methods for separating DNA fragments based on difference in conformation but independent of length would be of great interest. Such methods could be used for analyzing complex DNA samples containing many fragments of different length. We constructed a collection of tripartite 298 bp DNA fragments containing all 8 single-base mismatches, 5 C-bulges ranging from 1 to 5 nucleotides, and the 3 major UV-induced DNA lesions. This collection was used to develop a 2-D PAGE electrophoresis method for separating DNA fragments based on length and conformation in the 1st dimension and on length in the 2nd dimension. Most DNA fragments with altered conformation had reduced migration rates due to curving. This curving was minimized during 2nd dimension electrophoresis by introducing intercalators or using denaturing conditions. This resulted in DNA fragments with altered conformation migrating in front of an arc comprising fragments of different length but regular conformation. We noted a relationship between the degree of alteration in conformation and separation. DNA fragments containing from 1 to 5 C-bulge showed progressively increased separation. In contrast, DNA fragments containing single-base mismatch did not resolve. Applications of this simple and robust method includes simultaneous mismatch scanning of multiple fragments for length polymorphisms, isolation of damaged DNA molecules from intact molecules, and estimation of reannealing efficiency after hybridization of complex DNA samples.

Comparison of Three Different Amplification Protocols on Small Amounts of Total RNA for Microarray Analysis. *L.M. Barnes, C.D. Wolfgang, C. Lavedan.* Pharmacogenetics, Novartis Pharmaceuticals Corp, Gaithersburg, MD.

The introduction of DNA microarray technology has provided a powerful tool for genome-analysis of RNA.. This technology is of particular interest to the Pharmaceutical Industry because of its capability to provide a molecular signature of drug action that may lead to information about drug efficacy and toxicity. There are several different DNA Microarray platforms available. Our laboratory uses the Affymetrix GeneChip System. The current protocol from Affymetrix requires a minimum of 5g purified total RNA starting material. However, this valuable tool cannot be used for studies where small biopsies are harvested because they do not yield large amounts of RNA. The purpose of this study was to survey three different amplification protocols (Phillips and Eberwine et al 1996, Wang et al 2000, and Van Gelder et al 1987) to determine if the profiles generated by them are similar to the profile generated by the Affymetrix protocol. For this study, samples of total RNA from a breast tumor were processed using the standard Affymetrix protocol. Dilutions of this sample were later amplified using the three different methods described above. All samples were hybridized to the Affymetrix human microarrays (HuGeneFL and Human U95Av2) which contain probe sets for 6,000-12,000 genes and ESTs respectively. The number of genes detected on these arrays varied between 7% (Wang et al 2000 protocol) to 37% (Affymetrix standard protocol) using the Affymetrix algorithm. Direct comparison of the profiles from the amplified samples indicates that there were greater than 1,000 genes different from the profile of the sample processed using the standard Affymetrix protocol. An inspection of the housekeeping genes beta-actin and GAPDH showed significant variation compared to the unamplified sample. Similar differences were detected in many other genes when a similar comparison was done. The finding from this study suggests that amplification of total RNA may alter the global expression profile of a sample, warranting caution when using amplified sample data to make inferences about mechanism of action, efficacy and toxicity.

A rapid, simple and automatable DNA purification system for diverse sample types. *L. Flanagan, P. Mandrekar, R. Bitner, S. Koller, A. Tereba.* Genetic Analysis, Promega Corporation, Madison, WI.

With the advent of cloning, PCR amplification and sequencing the human genome, DNA analysis is now able to answer diverse questions in cell regulation and disease development. With these advances, reliable extraction of DNA from various sources has become important in basic and clinical research. Many methods currently involve the use of hazardous chemicals, are labor intensive and require different approaches for different sample types. In addition, most purification schemes are not automatable. We report here a simple, rapid and automatable system that can handle a wide range of samples. Based on a paramagnetic particle that binds DNA in the absence of hazardous organic solvents, DNA is rapidly obtained, free of PCR inhibitors from samples like whole blood and urine. Using a Proteinase K pre-treatment, hair, bone and tissue, including formalin fixed paraffin embedded thin sections, are easily processed. The ability to obtain PCR amplifiable DNA from this latter sample type allows a correlation between pathology and molecular analyses. DNA purification from blood cards and buccal swabs is accomplished using a heating step and centrifugation followed by the standard purification protocol. For all sample types, both genomic and mitochondrial DNA is isolated.

To increase efficiency in the analysis of DNA, our paramagnetic particles are designed to bind a limited amount of DNA. Thus, for common reference samples the particles deliver a uniform amount of DNA regardless of the amount of DNA present in the sample. This eliminates the need to quantitate DNA and gives uniform results in DNA analysis. The amount of DNA recovered can be modulated between 50ng and 1ug. This DNA purification system has been automated on the Beckman Biomek 2000, F/X and Tecan Genesis 150 systems allowing for high throughput purification from diverse sample types.

DNA yields from buccal swabs: self-collection vs. nurse/nurse-supervised collection. *J. Chang, K. Heard, S. Roberts, K. Steinberg, M. Gallagher.* CDC, Atlanta, GA 30341.

Buccal cell collection is a noninvasive alternative to whole blood collection that provides means for obtaining DNA suitable for clinical and research studies. Self-collected buccal swabs can be returned by mail, making self-collection an economical approach particularly for collecting samples from a cohort of widely dispersed individuals. However, buccal swabs yield varying amounts of DNA, unlike whole blood samples where collection always involves a trained health professional. To assess different collection methods, we compared DNA yields from two groups: 95 self-collected mail-returned buccal swabs from adult female subjects in a case-control study of birth defects in the metro Atlanta area, and 119 nurse-collected (or nurse-supervised self-collected, referred to here as nurse-collected) buccal swabs from subjects in a case-control study of ischemic stroke in women in the Maryland-DC area. DNA was isolated using a rapid method yielding about 600 μ L of buccal cell lysate. Human genomic DNA was quantified by real-time quantitative PCR. Average total yields were 1.13 ± 1.34 mg (range 0-7.43, median 0.80) for self-collected brushes and 0.84 ± 0.91 mg (range 0.05-4.47, median 0.47) for nurse-collected brushes. No significant difference existed between average total DNA yields of self- and nurse-collected buccal brushes. However, when standard extraction volume was used, 21% of the self-collected samples had a concentration ≤ 0.10 mg/mL, but $<1\%$ of the nurse-collected samples did. Buccal samples with concentrations ≤ 0.10 mg/mL are problematic when amplified by standard PCR methods, and allele dropout may occur. In some cases, DNA concentrations may be increased using additional steps that concentrate the DNA lysate, but this may limit the extent to which the sample can be used in future studies. Though collection of buccal swabs by mail is convenient and economical, the likelihood of obtaining samples with low DNA concentration may have significant impact on downstream applications. Thus, the method of collection should be carefully considered.

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Novel approaches to visualizing and analyzing array-based comparative genomic hybridization data. *J. Greshock, T.L. Naylor, S.H. Cleaver, M.R. Gererro, A. Margolin, M.L. Liebman, B.L. Weber.* University of Pennsylvania, Philadelphia, PA.

Recent advances in microfabrication and genome sequence assembly facilitate accurate measurements of genomic gains and losses using array-based comparative genomic hybridization (aCGH). Defining disease-related genomic changes in human tissue at a high resolution is relatively novel unto itself and efforts to standardize aCGH data analysis and to develop tools for data visualization are limited. We have developed several software packages devoted to array CGH analysis and data visualization that are downloadable as a part of a larger on-line resource for array CGH development and analysis. The CGH Browser experiment viewer plots clone ratios vs. genomic location by chromosome. Unlike typical methods for generating these plots, the CGH Browser can handle multiple experiments simultaneously (including dye swap analysis), allow users to annotate experiment results, export results to files or databases, and ultimately link mapped clones to several on-line line genome feature databases (e.g. NCBI MapViewer and Ensembl). The CGH Circle Viewer is another downloadable visualization tool that efficiently displays an entire CGH experiment in a single circular format and allows overlay of data from multiple experiments, identifying common gains and losses. In addition to these CGH analysis tools, we have developed novel means of visually integrating mapped genome features and their associated annotations with aCGH data in the circular format. One can effectively link an aberrant region of the genome seen in an aCGH experiment to features on a physical map (e.g. Unigene genes, STSs, etc). We will demonstrate all these tools using data from a melanoma tumor progression series as a model. Standardizing statistical methods and creating publicly available tools devoted to CGH data analysis will greatly facilitate data exchange and regiment a language of analysis. This is of particular importance because of the increasing volume of aCGH in public data repositories and the need to integrate expression data and genome map features with aCGH data.

Automated Nucleic Acid Purification in DNA Banking. *K. Heard¹, E. Heath², B. Carr², L. Wöhr¹, D. O'Brien², K. Steinberg¹, M. Gallagher¹.* 1) CDC, Atlanta, GA; 2) Genra Systems, Minneapolis, MS.

The National Health and Nutrition Examination Surveys (NHANES) are conducted by the CDC to assess the health of the U.S. population. In anticipation of advances in genetic research, a DNA bank was established in 1988. In the current survey, whole blood is being collected for DNA isolation. This type of specimen also affords the opportunity for the separation of plasma that can be analyzed for toxins, heavy metals, and micronutrients. The purpose of this study was to determine the feasibility of using a combination of workflow management and automated instrumentation to process these samples in a high throughput laboratory environment. We investigated 1) separating whole blood into plasma and packed cells, which could be stored frozen during peak periods and 2) incorporation of laboratory automation for DNA processing. Packed cells were prepared from 1,444 paired whole blood samples (8 ml) collected in ACD anticoagulant from 722 adult subjects. The samples were stored at -80C until processing on the Autopure LS (Genra Systems, Inc.), an automated nucleic acid purification instrument. DNA yield and purity were determined and reproducibility was assessed by comparing the paired samples. Samples with DNA yields of <50ug were scored as invalid, and of the 1,444 packed cell samples, 19 (1.3%) were invalidated and omitted from the analysis. The results showed that the average DNA yield was 204 ug (range 50-610ug) and the average UV absorbance ratio A260/A280 was 1.98. Comparison testing showed that 82% of the paired samples differed by less than 50 ug. We also compared the DNA yield and purity of samples prepared using automated vs. manual processing. Sixteen blood samples were collected in duplicate: one set was purified using the AUTOPURE LS and the other was purified manually using Puregene reagents. The results showed the two sample sets to be highly correlated and the DNA yield and purity were comparable between the two methods. This investigation demonstrated that large quantities of high quality DNA could be obtained from frozen packed cells using an automated process, providing a means to increase throughput, improve workflow, and reduce human error.

Effect of Multiplex PCR Conditions on the SNPstream® UHT Genotyping System. *M. Mody¹, J.C. Myler¹, M.J. Thomas², S. Varde¹, C.A. Gelfand¹.* 1) Applications Research, Orchid BioSciences, Inc., Princeton, NJ; 2) DNAPrint genomics, Sarasota, FL.

Single Nucleotide Polymorphism (SNP) genotyping has important implications in the fields of gene mapping, pharmacogenetics and drug discovery as scientists investigate the relationship between gene structure and function and the effects of gene variation in disease and drug development. Orchid BioSciences, Inc. has developed an ultra high-throughput system called the SNPstream UHT Genotyping System that is cost-effective, reliable and accurate. The assay utilizes multiplex PCR followed by multiplex SNP-IT™ tag array, Orchid's next-generation proprietary single base primer extension technology. We have investigated the effect of different multiplex PCR conditions on SNP genotyping using the SNPstream UHT Genotyping System. Two marker panels of 12 SNPs (24 total) were each assayed with three multiplex PCR conditions, including a ramp-up protocol, a touchdown protocol and a standard cycling protocol with a constant annealing temperature. Initial findings suggest that all three methods worked equally well in the SNP-IT assay, indicating the robustness of the system and the flexibility of the multiplex PCR conditions for SNP analysis on the SNPstream UHT Genotyping System.

Gene expression in a partial trisomy 16 animal model of Down syndrome. *R. Lyle, C. Hendrichsen, C. Gehrig, S. Deutsch, S. Antonarakis.* Dept Medical Genetics, Centre Medicale Univ, Geneva, Switzerland.

Down syndrome (DS), caused by trisomy for chromosome 21 (T21), is the most common genetic cause of mental retardation with an incidence of approximately 1/700 live births. In addition to the mental retardation and facial characteristics, there are many other phenotypes, including congenital heart disease, early onset Alzheimer's disease and an increased risk of leukemia. With the development in recent years of mouse models of DS, and the completion of the chromosome 21 sequence, we are poised to gain a greater insight into the underlying molecular biology of DS. The presence of an extra copy of certain but not all of the approximately 225 HC21 genes are predicted to contribute to aspects of the complex DS phenotype. It is often assumed that all genes from the extra chromosome 21 will be expressed at a level 1.5x relative to diploids. However, since regulation of gene expression is complex, involving regulatory loops, many genes may not conform to this prediction. Identifying which genes are overexpressed, and to what levels, is obviously important to understand genotype-phenotype correlations in DS. The development of microarray technology has allowed the simultaneous examination of expression levels of many genes to be compared across tissues. However, there are certain limitations to microarray experiments: sensitivity and dynamic range of detection, large RNA quantities required. We have used a high-throughput real-time quantitative PCR (qPCR) assay to examine the expression level of 115 genes (including 82 HC21 orthologues) in the Ts65Dn mouse model of DS. Tissues examined so far include brain, heart, fibroblasts, and blood. The qPCR assay has enabled us to detect genes which are overexpressed, and those which are not, in Ts65Dn thus helping to identify those genes contributing to the DS phenotypes.

Towards assembling genome-on-a-chip using DNA representation array. Z. Yuan¹, K. Zhang^{1,2}, R. Zhang³, D. Yoon³, J. Jeong³, X. Chen⁴, J. Korenberg⁴, S. Shearer⁵, B. Czerniak³, L. Jin¹ and *The first two authors have equal contribution to this work.* 1) Center for Genome Information, University of Cincinnati, Cincinnati, OH; 2) Human Genetics Center, University of Texas-Houston, Houston, TX; 3) Department of Pathology, MD Anderson Cancer Center, Houston, TX; 4) Department of Pediatrics, Cedars-Sinai Medical Center, Los Angeles, CA; 5) Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX.

The integration of CGH and microarray technology provides a high-resolution and quantitative tool to identify copy-number variation across the genome. The existing array-based methods for CGH are generally marred by laborious DNA preparation and purification procedures, which make them practically difficult to be scaled up. So far, even the highest density CGH-array provides less than 10% genome coverage, and leaves genetic imbalance in the rest 90 percent of genome undetected. We report a novel approach to generate genome-scale CGH arrays for the detection of gene copy number imbalance in a variety of diseases. A species-specific whole genome amplification method, which we refer to as human-specific SOGA, enables the preparation of sufficient amounts of array probes (DNA representation) by PCR using bacteria lysates instead of purified DNA as template. By avoiding laborious procedures of large-volume bacteria culture, plasmid isolation and contamination checking, the labor and cost of making CGH arrays can be reduced by 10~100 folds, and implemented in semi-automatic manner. Furthermore, the species-specific amplification also removes efficiently the repetitive structures in the genome and reduces the background noise drastically with both high sensitivity and high specificity. This approach makes possible the realization of a genome-on-a-chip approach, which requires arraying approximately 20,000 BAC clones covering the whole human genome on a single microscope slide, and will promote the clinical application of array-based CGH technology as a powerful diagnostic tool.

RT-coupled 5' nuclease assay — A general and high throughput method for detection of gene differential allele expression using transcribed SNPs as endogenous reporters. *G. Zhu¹, R.H. Lipsky¹, K. Xu¹, S. Ali¹, T. Hyde², J. Kleinman², L.A. Akhtar¹, D.C. Mash³, D. Goldman¹.* 1) NIAAA, NIH, Rockville, MD; 2) NIMH, NIH, Bethesda, MD; 3) Department of Neurology, University of Miami, Miami, FL.

Detection of unequal expression of maternally and paternally derived gene copies depends on the availability of gene polymorphisms in transcribed sequences. Single nucleotide polymorphisms (SNPs) represent the most common type of sequence variation and are frequently found in transcribed regions. Such SNPs are endogenous reporters for detecting differential allele expression in heterozygous individuals. To facilitate detection and functional analysis of cis-acting regulatory alleles and imprinting, we developed a simple and high throughput quantitative method, designated the RT-coupled 5' nuclease assay, for measuring allele expression ratio. We chose human COMT as a model gene. A transcribed SNP, rs-4818(C>G) [dbSNP], was used as maker for detecting allele ratio. Heterozygous individuals for rs4818(C>G) were identified through genotyping genomic DNAs by high throughput 5' nuclease assay using two allele-specific fluorescent-labelled probes. Total RNAs were isolated from these individuals and reverse transcription (RT) was performed. 5' nuclease assay was performed again but using the cDNA as the template instead of genomic DNA. Heterozygous genomic DNA was used as 1:1 allele ratio control. Fluorescence signal was collected after each cycle of amplification and allele ratio was calculated from the adjusted difference in threshold amplification cycle between the two alleles. Using this method, we discovered differential allele expression of human COMT both in lymphoblastoid cell lines (17/22) and postmortem cerebellar cortex samples (13/15), with one allele consistently more highly expressed than the other, ranging ratios from 1.5 to 4. These results were verified using quantitative RFLP of RT-PCR products. In summary, RT-coupled 5' nuclease assay is a simple, rapid, and reliable general method for quantitating differential allele expression, and can thereby greatly aid in the detection and evaluation of cis-acting functional DNA polymorphisms potentially in any gene and genomic imprinting.

PCR system to reduce allele drop out and preferential amplification in genotyping low concentration/low quality DNA samples. *S. Roberts, J. Chang, K. Heard, K. Steinberg, M. Gallagher.* Centers for Disease Control, Atlanta,GA.

Although allele drop out(ADO) and extreme preferential amplification(EPA) are well-studied phenomena in PCR amplification of single cells, much less attention has been paid to their roles in genotyping low concentration/low quality DNA samples, e.g. DNA from blood spots or buccal swabs. Increasing denaturation temperature and denaturation/annealing times, decreasing cycle number, and use of alternate polymerases have previously been shown to decrease ADO/EPA in single-cell PCR. We examined the effects of the following modifications on ADO/EPA in low concentration/low quality DNA samples. Human genomic DNA was quantified using real-time quantitative PCR. Three PCR protocols were evaluated for amplification of microsatellite marker D13S317: 1)TaqGold protocol, 2)TaqGold/modified cycling, and 3)Roche Expand High Fidelity PCR System/modified cycling. Low concentration samples consisted of diluted DNA isolated from whole blood. Low quality samples consisted of buccal cell DNA samples (0.17-2.2 ng/uL). Fragment analysis was performed using the ABI 3100. ADO, EPA, peak height ratios, and overall quality of results were analyzed. Using 25 pg of high quality template, ADO occurred only in protocol 3 (15.8% of samples). EPA occurred in protocols 1 and 3 (15.8% and 20% of samples). Using 12.5 pg, the ADO rate was observed in 60% of samples amplified using protocols 1 and 2 and 27% of samples using protocol 3. EPA also increased overall, occurring in 14.3%, 10%, and 10% of samples for protocols 1, 2, and 3, respectively. Amplifications done on low quality templates showed ADO and EPA in 5.5% of samples for protocol 1 and in no samples amplified by protocols 2 and 3. In conclusion, recommendations made to minimize ADO/EPA in single-cell PCR can also be effective in the amplification of low concentration/low quality DNA. Using alternate polymerases decreased ADO at extremely low concentrations. For moderately low concentration samples, the best results were achieved using TaqGold with modified cycling. When using low quality samples, modifying PCR conditions decreased ADO and improved peak balance, regardless of the type of polymerase used.

Program Nr: 1350 from 2002 ASHG Annual Meeting

High Throughput Wizard® SV 96 Genomic DNA purification from human buccal swab samples. *T. Worzella, C. Cowan, T. Grunst, K. Hooper.* Promega Corp., Madison, WI.

Purification of genomic DNA from human buccal samples is challenging and difficult to automate for high throughput processing. Here we demonstrate a high throughput, fully automatable, genomic DNA purification procedure from buccal swab samples. Genomic DNA is purified using Promega's Wizard® SV 96 Genomic DNA Purification System either manually or by using an automated liquid handler such as a Beckman Biomek® FX. Purified genomic DNA is high quality and suitable for downstream applications such as PCR.

Program Nr: 1351 from 2002 ASHG Annual Meeting

Molecular haplotype determination using Pyrosequencing™ technology. *M. Pettersson, M. Bylund, A. Alderborn.*
Pyrosequencing AB, Vallongatan 1, SE-752 28 Uppsala, Sweden.

The identification of haplotypes from a combination of single nucleotide polymorphisms (SNPs) on one chromosome is a powerful tool for genetic research. Haplotyping is usually performed statistically by computational analysis, or by time consuming cloning techniques. Here we present a simple molecular approach for reliable haplotype determination on an individual basis. The procedure is based on allele-specific PCR (AS-PCR) in combination with Pyrosequencing™ technology.

AS-PCR primers for each allelic variant of a SNP, with their 3' ends located at the SNP position, were used. A mismatch introduced in the second base from the 3' end was shown to dramatically improve allele-specificity. Analysis of multiple SNPs on amplified fragments using Pyrosequencing technology allowed determination of haplotypes. Genotyping of heterozygote positions after AS-PCR gave typical homozygous patterns, where the identity of the present allele depended on the allele-specific initial amplification.

Haplotype determination by the described procedure proved to be highly reliable. The results obtained by Pyrosequencing technology have the benefit of being quantitative, enabling detection of any non-specific allele amplification. In addition, the possibility of multiplex SNP genotyping using Pyrosequencing technology makes the described method an efficient solution for molecular haplotyping.

High Throughput Genotyping using a Microfluidic System that Integrates, Reagent Assembly, PCR, Homogeneous Phase Detection and Data Analysis on Nanoliter Volumes. *C. Peponnet, V. Phillips, C. Brooks, J. Coffin, Y. Yurkovetsky, J. Lai, J.A. Ferguson, E. Li, M. Strachan, A.R. Kopf-Sill.* Caliper Technologies, Mountain View, CA.

Numerous studies are performed in an attempt to understand the correlation between disease risks or drug response and individual genetic variations such as single nucleotide polymorphisms (SNPs). These association studies often necessitate millions of genotypes (thousands of patients X thousands of SNPs). Current study sizes are limited by the absence of a sufficiently low cost, high throughput genotyping platform. The microfluidic system that we are developing for genotyping integrates the reaction assembly, the PCR, the homogenous detection and the data analysis using only nanoliter reaction volumes. DNA samples are sipped from a microplate through a capillary connected to a channel inside a microfluidic chip. The sipped sample while flowing through the main channel is mixed with polymerase, dNTPs and MgCl₂ coming from a side channel. This sample/reagent mixture is then split equally into 8 independent channels whereupon 8 different SNP specific reagents (ie. primers and probes), are added -1 per channel. These 8 complete PCR mixes continue to flow through the thermocycling zone of the channels. The reactions then pass the detection zone and flow to the waste wells. The presence or absence of each allele is determined by measuring the fluorescence emission signal for two fluorescent reporters (one per allele) resulting from a 5 nuclease assay. The data is then automatically processed and genotypes are called. Samples are sipped serially, one after another, while they are genotyped on 8 SNPs in parallel, like on an assembly line. The reaction transit time from sample to detection, is approximately 25 min. DNA samples are sipped every 4 min. from a microplate and are separated by buffer spacers. Reaction volumes vary from 3 to 10 nanoliters and PCR concentrations are in the 100nM range. This microfluidic system, comprising instrument, chip and software offers a low-cost (very low volume) and high-throughput alternative to SNP identification (integrated system).

LNA nearest neighbor energy rules. *R.J. Peterson*¹, *P.M. McTigue*², *J.D. Kahn*². 1) Celadon Laboratories, Inc, College Park, MD; 2) Dept. of Chemistry & Biochemistry, University of Maryland, College Park, MD.

The industrialization of genomics demands DNA methods that provide accurate results on the first try. Primers and probes comprised solely of natural DNA may not suffice. Locked Nucleic Acid (LNA) is an RNA derivative that stabilizes hybridization, reportedly by 1.0° C to 5.0° C per LNA incorporation, due to more rigid structure and/or improved base stacking. Oligonucleotide primers and probes that incorporate LNA bases can be shorter and thus showed increased sensitivity and specificity. However, since no systematic assessment of sequence dependence has been performed, it has been impossible to predict accurate melting temperatures (T_m) for oligos of varied sequence and length, and for varied salt concentration. To address this deficiency, an extensive set of absorbance melting curves was obtained (using a Cary 100 spectrophotometer) on duplex 8-12 bp oligos, each with a single LNA modification in one strand (from PrOligo). Comparison to reference DNA allowed estimation of enthalpy and entropy increments (DDH° and DDS°) attributable to the LNA modification, for the entire set of 64 XLY perfect match triplets, where X and Y are DNA bases and L is a LNA base. A salt correction factor to adjust DS° was found to differ between LNA and DNA. In combination with energy rules for DNA, this data set allows T_m prediction for arbitrary LNA:DNA hybridizations. As is the case for DNA, LNA energy rules are strongly sequence dependent. The average T_m increases were as follows: LNA-A: 1.9+/-1.6° C; LNA-G: 3.0+/-2.1° C; LNA-T: 3.6+/-1.2° C; and LNA-C 4.4+/-1.7° C, with standard deviations reflecting sequence context. The effect on oligo T_m varied from -0.5° C to 7.8° C. Surprisingly, both DDH° and DDS° were generally positive, suggesting that LNA:DNA stability is due primarily to decreased single-stranded flexibility, not to improved base stacking. This work shows that sequence-dependent energy rules are likely to be essential for robust prediction of LNA primer and probe T_m for varied sequences, lengths, and salt concentrations. LNA:DNA design rules have been incorporated into Celadon's primer design package, Probity.

Program Nr: 1354 from 2002 ASHG Annual Meeting

Automation of genomic DNA purification from cell cultures on Beckman Coulter's Biomek® 2000 laboratory automation workstation and Biomek FX liquid handling system using Promega's Wizard® Genomic DNA

Purification kit. *K. Roby*¹, *H. Wai*¹, *M. Cu*¹, *D. Campbell*¹, *T. Grunst*², *D. Kephart*². 1) Automated Solutions Dev Ctr, Beckman Coulter, Inc, Fullerton, CA; 2) Applications, Promega Corporation, Madison, WI.

With the advent of the post-genome era comes the need for fast and elegant methods for purification of genomic DNA, that embrace the simplicity of automation and the robustness of PCR¹-based analysis such as forensics, genotyping, HLA typing, and SNP detection. We present here a rapid fully automated method for the isolation of genomic DNA using the Promega Wizard Genomic DNA Purification Kit on Beckman Coulter's Biomek 2000 Laboratory Automation Workstation and Biomek FX Liquid Handling System. Genomic DNA is bound to the Wizard SV 96 DNA binding plates, which makes the process simple and easy to implement in the DNA research laboratory. Evaluation of the purified DNA was done by analysis of the generated PCR products. Four MapPairs[®] were used from the microsatellite marker set available from ResGen@ Invitrogen Corp. The generation of labeled PCR products was achieved using the microsatellite marker set labeled with Beckman Coulter's WellRED Dyes. These products were then run on Beckman Coulter's CEQ[™] 8000 Genetic Analysis System. Automation logistics (i.e. labware, timing and robotics control) are also discussed. [®]All trademarks are property of their respective owners. ¹PCR is covered by patents owned by Hoffman-La Roche, Inc.

Fine-mapping of segmental duplications on human chromosome 7. *J. Cheung, J.R. MacDonald, R. Khaja, A.K. Hudek, L-C. Tsui, X. Estivill, S.W. Scherer.* The Centre for Applied Genomics, Dept. of Genetics, The Hospital for Sick Children, and Dept. of Molecular and Medical Genetics, University of Toronto, Canada.

The presence of segmental duplications of DNA (duplicons) in the human genome can predispose and cause the occurrence of chromosomal rearrangements that are sometimes associated with disease (often called genomic disorders). In an effort to further annotate human chromosome 7, we have performed in silico detection and FISH experiments aimed at identifying the precise locations and orientations of all large (≥ 10 kb) and nearly identical ($\geq 90\%$) segmental duplications. We have generated a high quality DNA sequence map of human chromosome 7 (HSC7) by combining both public and Celera sequences. The resulting HSC7 assembly consists of 154Mb of sequences with four gaps (two sequence gaps and two physical gaps). The sequence was masked for repeats and then compared against itself using MegaBlast2. The results were initially parsed under these criteria: $\geq 90\%$ sequence identity, ≥ 80 bp in length, and expected value $\leq 10^{-30}$. In addition, identical hits (same coordinate alignments) as well as mirror hits (reverse coordinate alignments) were removed. Transchromosomal duplication analysis was performed against the NCBI build 29 genome assembly using the same criteria. Contiguous modules of duplicated sequences with size over 10kb were kept in the analysis. These results were subsequently converted into a coordinate file as an input for display using GenomePixelizer. As well, Blast was performed against a local Genbank NR database to identify clone and gene names that match to these duplicated regions. Overall, 5% (8/154 Mb, 190 modules) of chromosome 7 is composed of duplicated sequences (4% intrachromosomal and 2% transchromosomal), with an average size of 40kb and 94% sequence identity for each duplicon. Moreover, several large duplicons were identified in 7p22, 7p14/15, 7q11.21, 7q22, 7q36 as well as the pericentromeric region. Many of these duplications, showing chromosomal and temporal specificity, vary greatly in size, content, and organization. The characterization of these regions will allow us to examine their potential involvements in genomic disorders.

Duplication-mediated rearrangements detected by array comparative genomic hybridization. *D.P. Locke¹, R. Segraves², L.D. Carbone³, M. Rocchi³, S. Schwartz¹, R.D. Nicholls⁴, D.G. Albertson², D. Pinkel², E.E. Eichler¹.* 1) Department of Genetics, CWRU, Cleveland, OH; 2) Cancer Center, UCSF, San Francisco, CA; 3) Instituto di Genetica, Universit di Bari, Bari, Italy; 4) Department of Psychiatry, UPenn, Philadelphia, PA.

Array comparative genomic hybridization (CGH) is a high throughput method of detecting copy number changes between genomes, allowing simultaneous assessment of thousands of genomic loci. We utilized a human BAC array composed of 2,460 target sites, representing ~360 Mb of sequence distributed across the genome, to assess copy number difference between Asian and African great ape species as well as isolated human populations. A subset of the array CGH findings were verified by comparative FISH and molecular analyses. A total of 58 events were ascertained in the comparison of pygmy chimpanzee, common chimpanzee, gorilla and orangutan. Lineage-specific large-scale rearrangements, as well as events shared between species were detected. Interestingly, 60% of the verified rearrangements occurred within close proximity to segmental duplications. Compared to prior cytological and karyotypic analyses, these results suggest that hominoid genomes have been subjected to considerably more microdeletion and microduplication than expected. To complement these studies we developed a smaller-scale array focused on a region of the human genome rich with segmental duplications, 15q11-q13. We have tested the potential of array CGH to detect large-scale genomic rearrangements in this region in a variety of human clinical samples. Our results show this smaller array accurately detected both gains and losses across this region in a reproducible manner. Recent computational analysis of segmental duplications within the human genome identifies 169 regions of potential genomic instability (Bailey et al., 2002). These regions will be targets for future comparative array CGH analysis, as well as potential sites of large-scale variation and genomic disease within the human population. Based on these analyses, targeted array CGH is an extremely effective technique for detecting genomic imbalance in both comparative and clinical settings.

Genome architecture, low-copy repeats and genomic breakpoints in 17p. *P. Stankiewicz*¹, *J. Dapper*¹, *C. Shaw*¹, *S.-S. Park*¹, *L. Potocki*¹, *J.R. Lupski*^{1,2,3}. 1) Dept. of Molecular & Human Genetics; 2) Pediatrics, Baylor College of Medicine, Houston, TX; 3) Texas Children Hospital, Houston, TX.

Over 90% of SMS patients are deleted for an identical ~4 Mb genomic region in 17p11.2, whereas in the remainder both smaller and larger sized deletions were identified. The recurrent common deletions and reciprocal duplications dup(17)(p11.2p11.2) occur via non-allelic homologous recombination utilizing flanking low-copy repeats (LCRs), termed proximal (~256 Kb) and distal (~176 Kb) SMS-REPs as substrates. A third LCR copy, middle SMS-REP (~241 Kb) maps between them and is inverted in orientation. In addition, portions (~10-25 Kb) of the SMS-REPs were identified along the entire chromosome 17, on 17p13.1, 17p12, 17q11.2, 17q12, 17q21.2, and 17q23.2. To further investigate the relevance of SMS-REPs structures to chromosome rearrangements, using FISH with BACs, PACs, and long-range PCR products and PCR on somatic cell hybrids we analyzed the breakpoints of unusual sized deletions in eleven patients with SMS and six patients with chromosome translocation breakpoints involving 17p11.2. The analysis of five breakpoints of larger sized deletions led to the identification of novel, large LCRs: LCR17pA (~410 Kb), LCR17pB (~210 Kb), LCR17pC (~90 Kb), and LCR17pD (~150 Kb). Smaller sized deletions enabled us to narrow down the SMS critical region to ~1.1 Mb, and possibly to ~210 Kb. Interestingly, five chromosome breakpoints involved the inverted middle SMS-REP. Two 17p11 translocation breakpoints were found to be located within the centromere, two within ~0.5 Mb segment from cen17 and one within the distal SMS-REP. Four out of six partner chromosome breakpoints were mapped within the most telomeric sub-bands: 1p36.3, 2p25.3, 10q26.3, and Xp22.3. Our data further supports previous observations that (peri)centromeric and subtelomeric chromosome regions together with higher order genomic architecture involving low-copy repeats play a significant role in origin of different genome rearrangements.

Gene expression via DNA microarray of anterior lens epithelium from patients with and without

Pseudoexfoliation Syndrome(PEX). *K.F. Damji^{1,2}, L. Wang¹, S. Xiao²*. 1) Eye Institute, University of Ottawa, Ottawa, ON, Canada; 2) Ottawa Health Research Institute, Ottawa, Ont, Canada.

Purpose: To compare the mRNA expression pattern from lens epithelium in patients with and without PEX.

Methods: Presence or absence of PEX was determined preoperatively by dilated, slit lamp examination. The lens capsule was removed during capsulorhexis at the time of phacoemulsification cataract extraction (PCE), collected and snap frozen to 80C. The experimental group consisted of 30 pooled lens capsules from PEX patients. The control group contained the same number of capsules from patients over age 60 without PEX. RNA was extracted from the capsules using RNeasy Mini kit from QIAGEN. The quality of RNA was tested with the Agilent Bioanalyzer. The Affymetrix microarray was run for both groups by Expression Analysis (EA), Durham, North Carolina. **Results:** The anterior lens capsule, removed during PCE, contained a confluent layer of lens epithelium by light microscopy. 10ug total RNA was obtained from the pooled capsules in each group. Total output genes from the microarray are more than 20,000. Two fold or more over expression in PEX vs. control was found in 16 genes. Conversely, there were 6 genes showing 2-fold or more under expression. The balance of genes showed either equal or slight under or over expression, in both groups.

Conclusion: We have demonstrated that it is possible to compare lens epithelial gene expression in PEX and normal controls via DNA microarray technology. PEX showed significant over or under expression of genes vs control. Further study needs to be done to confirm these differences. **Acknowledgements:** Technical assistance: Feisal Adatia, Sanjoy Gupta, May Griffith and her lab staff; Contributing surgeons: Sherif El-Defraway, George Mintsioulis, William Rock, William Hodge, Ralf Buhrmann; Eye Institute operating room nurses.

***In silico* discovery of gene coding and regulatory variants in mouse QTLs using strain-specific genome sequence databases.** *J.M. Sikel*¹, *K. Marshall*¹, *S. Burgers*¹, *E. Godden*¹, *F. Yang*¹, *K. Buck*², *M.F. Miles*³. 1) Human Medical Genetics Program, Univ Colorado Health Sci Ctr, Denver, CO; 2) Oregon Health Sciences University and VA Medical Center, Portland, OR; 3) Virginia Commonwealth University, Richmond, VA.

We have developed a high throughput pipeline for the *in silico* discovery of gene variations that lie within mouse QTLs. To identify coding region sequence differences the coding sequences of QTL localized genes are retrieved from strain-specific whole genome sequence databases and altered genes identified by *in silico* sequence alignment. To test this approach we first focused on two C57BL/6J (B6) x DBA/2J (D2) QTLs involved in chronic ethanol withdrawal severity (physical dependence) that lie on chromosomes 1 and 13. The sequences for 199 QTL-localized genes from these strains were aligned and interstrain variations directly identified *in silico*. Sixteen QTL genes were found that had altered coding regions, several of which represent promising candidates to underlie these QTLs.

To identify regulatory changes that may underlie QTLs high-density microarray studies of selected strains are used to identify genes differentially expressed between strains and the regulatory sequences of genes that map to QTLs are then compared between strains *in silico* to identify altered transcriptional control sites. To demonstrate this an 11,000 gene Affymetrix chip (U74Av2) was probed with B6 and D2 prefrontal cortex brain mRNAs and 165 differentially expressed genes were identified, 26 of which mapped to known B6xD2 alcohol-related QTLs. One of these genes had a 4-fold higher expression level in B6 compared to D2, was known to be involved in alcohol action, and had a regulatory sequence variation that, in B6 mice, completely disrupted the core binding site for *Ikaros*, a known transcriptional repressor. Finally, the above approaches have wide general utility, and should be applicable to any of the several hundred QTLs that have been identified using strains for which relatively complete genome sequences are now available. This work was supported by NIH grants AA11853, AA03527 (JMS), AA1114, AA13433 (KJB) and AA13200 (MFM).

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Gene Expression Assays To Enable High Throughput Genomics. *K.E. Rogers, K. Lazaruk, M. Rhodes, C. Walworth, E. Spier, F. de la Vega, D. Gilbert.* Genomic Assays Business, Applied Biosystems, Foster City, CA.

The draft of the human genome enables scientific discoveries in many fields. The challenge is to make this wealth of genome information available to the laboratory.

Real time PCR using 5 nuclease chemistry with Taqman® MGB probes is the gold standard for gene expression studies. Experimental setup is simple consisting of addition of two reagents (assay mix and universal master mix) to the Target DNA. Applied Biosystems has launched two complementary product offerings that together provide a complete solution for ready to use assays for gene expression studies. These assays use the 5 nuclease chemistry with Taqman® MGB probes. All assays are provided in an easy to use single tube format.

The Assays-on-Demand™ gene expression products, will provide a validated assay for every Human gene. The initial release set is 12,000 assays targeted at all the known Refseq transcripts. Assays can be selected via gene name, NCBI reference or functional attributes. To complement the pre-designed assay products the gene expression Assays-by-DesignSM service, allows customers to submit a target sequence of interest from any organism. Applied Biosystems uses proprietary algorithms to design an assay for each target. Following synthesis and formulation, the assay is analytically tested for integrity before shipping to the customer. Genomic assays provided by both the Assays-by-DesignSM service and Assays-on-Demand™ products are delivered in an easy to use single tube format. Experimental setup is minimized consisting of addition of two reagents (assay mix and universal master mix) to the Target cDNA. Details on the design, production and testing of these assays will be presented.

Differential gene expression patterns identified in Sjogren's Syndrome patients using microarray analysis. *M.E. Grandits, D.B. Machado, C.M. Meyer, E.C. Baechler, K.M. Hughes, S.J. Lippert, A.N. Leiran, J.A. Ermer, W.A. Ortmann, P.M. Gaffney, T.W. Behrens, N.L. Rhodus, K.L. Moser.* University of Minnesota, Minneapolis, MN.

Sjogren's syndrome (SS) is a chronic heterogenous autoimmune and lymphoproliferative disorder primarily affecting salivary and lacrimal glands. The molecular events that lead to SS are poorly understood but thought to involve multiple components of immune dysregulation. The goal of this study was to identify genes that are aberrantly expressed in blood cells of patients with SS. We have used microarray analysis to compare genomic-scale gene expression profiles of peripheral blood mononuclear cells (PBMCs) in 22 SS patients and 23 healthy controls. All patients fulfilled the 2002 Revised European Criteria for Classification of SS recently proposed by the American-European Consensus Group. SS patients were thoroughly evaluated for exocrine and extraglandular manifestations. Total mRNA was isolated from PBMCs, reverse transcribed into cDNA, and used to generate labeled cRNA. The cRNA was then hybridized to oligonucleotide microarrays to determine the level of expression in over 12,600 known genes. Over 630 genes were found to be differentially expressed between patients and controls using t-tests with nominal significance of $p < 0.001$. Expression levels of over 80 of these genes differed by at least 2-fold. Dysregulation of pathways important in apoptosis, ubiquitin/26S proteasome degradation, lymphoproliferation, and responses to interferon were apparent in these data. Multiple genes in each pathway were expressed differentially, including many genes previously implicated in autoimmune processes such as FAS, Ro/SSA, several HLA loci, and BAFF. The genes identified in this analysis impart a comprehensive view into potential pathogenic mechanisms of disease and provide an important source of potential targets for development of novel diagnostic and therapeutic approaches.

Chromosome-wide discovery of imprinted and differentially expressed genes for disease study. *K. Nakabayashi*¹, *S. Minagawa*¹, *L. Bentley*², *L. He*¹, *R. Kapur*¹, *A.C. Smith*¹, *J.S. Bamforth*³, *M. Oshimura*⁴, *R. Weksberg*¹, *G.E. Moore*², *S.W. Scherer*¹. 1) The Centre for Applied Genomics, Hospital for Sick Children, Toronto, Ontario, Canada; 2) Department of Fetal and Maternal Medicine, Institute of Reproductive and Developmental Biology, Faculty of Medicine, Imperial College, London, UK; 3) Department of Medical Genetics, University of Alberta, Edmonton, Alberta, Canada; 4) Dept. of Molecular and Cell Genetics, Faculty of Medicine, Tottori University, Yonago, Tottori, Japan.

Genomic imprinting is a phenomenon whereby genes are differentially expressed according to their parental origin. Systematic discovery of imprinted transcripts will facilitate the identification of genes involved in diseases showing parent-of-origin effects (e.g., growth abnormalities and malignant tumors). To develop methodologies for rapid identification of imprinted and differentially expressed genes, initially we are using human chromosome 7 as a model. Three imprinted loci (*GRB10* at 7p12, *SGCE/PEG10* at 7q21.3, and *MEST* at 7q32) are known to reside on chromosome 7. Moreover, imprinted gene(s) on chromosome 7 may be involved in Russell-Silver syndrome based on the fact that ~10% of patients have maternal uniparental disomy of chromosome 7 (UPD7). We are using (i) somatic cell hybrids containing a paternal or maternal human chromosome 7 and (ii) lymphoblastoid cell lines established from patients having UPD7, as RNA sources for RT-PCR and microarray experiments (Affymetrix GeneChip Array) to screen for differentially expressed genes. Initially, we identified 17 genes mapped on chromosome 7 showing at least three-fold difference in expression level between paternal and maternal alleles. Three of these were the known imprinted genes, *SGCE*, *PEG10*, and *MEST*. To confirm the imprinting status of the remaining 14 candidate genes, we are using somatic cell hybrid assays and allelic expression analyses in human fetal tissues. Two new genes, one being a coding transcript at 7q21 and the other a non-coding antisense transcript at 7q32 (*MESTITI*) have already been identified to be imprinted. Experiments are underway to characterize all of the transcripts on human chromosome 7.

A whole-genome linkage disequilibrium SNP map and validated assay resource. *D. Gilbert, C. Scafe, Y. Wang, M. Laig-Webster, X. Su, R. Koehler, H. Avi-Izthak, J. Ziegle, L. Wogan, E. Spier, F. De La Vega.* Applied Biosystems, Foster City, CA.

We developed a set of 5' nuclease allelic discrimination assays to score single nucleotide polymorphisms (SNPs) with the aim of creating a reference map for use in candidate-gene, candidate region and whole-genome linkage disequilibrium (LD) mapping studies. The assays were validated by individually genotyping 90 DNA samples, 45 from African-American and 45 from Caucasian individuals, selected from the Coriell Human variation collection. Our goal is to define a set of >150,000 assays distributed across all the genes in the genome for SNPs of high heterozygosity in at least one population. Candidate SNPs were prioritized from the Celera RefSNP database which contains 4 million unique SNPs from combined Celera and Public sources, through a triage process that requires evidence of independent discovery of the minor allele. We selected SNPs on 27,007 Celera gene predictions, in a gene focused picket-fence with an average density of one SNP per 10 kb of gene length, including 10 kb upstream and downstream of the predicted gene boundaries. PCR primers and TaqMan® probes for the 5' nuclease assay were then designed by a software pipeline that picks oligonucleotide sequences and then screens the assays against the genome database for potential artifacts. Following genotyping of 90 individuals, the performance of each assay is benchmarked against stringent criteria for background signal, adequate signal generation, and specificity. Our validation results showed that 94% of the SNPs tested in the population panels were polymorphic and about 90% of the assays passed our stringent performance criteria. Of those, 86% have minor allele frequencies ≥ 0.1 in Caucasian panel and 84% in African-American samples. These figures represent an extremely high SNP validation rate, and an unprecedented yield of common SNPs useful in LD mapping. The individual genotypes being generated have enabled us to identify blocks of LD and survey the haplotype diversity across all gene regions of the genome for these populations. This information is being used to refine the SNP set coverage.

Program Nr: 1364 from 2002 ASHG Annual Meeting

SNP assays to enable high throughput genomics. *A.H. Dodge, D. Dailey, P. Dong, M. Rhodes, L. Smith, F. de la Vega, G. Spier, C. Walworth, D. Gilbert.* Genomic Assays Business, Applied Biosystems, Foster City, CA.

The draft of the human genome enables scientific discoveries in many fields. The challenge is to make this wealth of genome information available to the laboratory. The 5 nuclease chemistry with Taqman®MGB probes has many attributes that lend itself to analysis of Single Nucleotide Polymorphisms. It has a high throughput, with 90k genotypes possible in a 8hr day from a single ABI Prism®SDS 7900HT and low DNA input, (1ng/5ul). Experimental setup is simple consisting of addition of two reagents (assay mix and universal master mix) to the Target DNA

Applied Biosystems has launched two complementary product offerings that together provide a complete solution for ready to use assays for genotyping via SNPs. These assays use the 5 nuclease chemistry with Taqman®MGB probes. All assays are provided in an easy to use single tube format.

The Assays-on-Demand™ SNP Genotyping products, provide 150K ready-to-use assays to pre-validated Human SNP targets which customers may select by, SNP, gene or genomic location. Each assay has been validated on two populations.

To complement the pre-designed assay products the SNP Assays-by-DesignSM service will allow customers to submit a target sequence of interest from any organism. Applied Biosystems uses proprietary algorithms to design an assay for each target. Following synthesis and formulation, the assay is analytically tested for integrity before shipping to the customer.

Genomic assays provided by both Assays-by-DesignSM service and Assays-on-Demand™ products are delivered in an easy to use single tube format. Experimental setup is minimized consisting of addition of two reagents (assay mix and universal master mix) to the Target DNA.

Details on the design, production and testing of these assays will be presented.

Long Distance Linkage Disequilibrium in the Han Chinese and Mixe Amerindians in four genomic regions. *K.A. Mather¹, M. President¹, S. Easteal², J. Hollenbach¹, W. Klitz³, G. Huttley², G. Thomson¹*. 1) Integrative Biology Department, The University of California, Berkeley, CA; 2) The Australian National University, Canberra; 3) Children's Hospital of Oakland Research Institute, Oakland, CA.

Analysis of linkage disequilibrium (LD) in the CEPH population revealed a pattern consistent with the action of natural selection; a few large genomic regions with a high density of markers in significant LD. In ten regions of the genome, this clustered LD is greater than that found in the HLA (Human Leukocyte Antigen) region (Huttley et al. 1999). The high LD regions identified are likely to be experiencing selection in the CEPH, making them good candidates in a search for regions under selection in other populations. However, if selection pressures vary among populations, patterns of LD may differ between populations. In order to determine if the high LD regions identified in CEPH also exhibit high LD in other populations, we analyzed microsatellite LD. We examined four genomic regions (ranging in size from 5 to 9 Mb) on chromosomes 6 (the HLA region), 20 (a control region without high LD in the CEPH), 7 and 22 (two CEPH high LD regions) in two non-Caucasian populations: Mixe Amerindians (n=46) and Southern Han Chinese (n = 48). We find evidence for LD between markers separated by several Mb, distances which are much greater than the sizes of the recently described haplotype blocks (Daly et al. 2001, Jefferys et al. 2001). The Mixe population had more LD than the Han in all four regions, consistent with the theoretical expectation (Slatkin 1994) that small populations will have more LD than large populations which have expanded. The ordering of regions by amount of LD (as measured in a variety of ways, including proportion of loci in significant LD and the length of tracts of LD) is similar in the two populations. The regions on chromosomes 6 and 7 have the greatest amount of LD, while those on chromosomes 20 and 22 have the least. These results are consistent with similar selective pressures (as reflected in long distance LD patterns) among regions in the two populations. This work is supported by NSF-DDIG, NIH grants and an IB summer fellowship to KAM. KAM is an HHMI Predoctoral Fellow.

Comprehensive setting of 30,000 polymorphic microsatellite markers throughout human genome. *A. Oka¹, K. Okamoto^{2,3}, S. Makino², T. Endo⁴, H. Hayashi², K. Fujimoto², E. Tokubo², A. Takaki², Y. Nagatsuka², T. Imanishi¹, T. Gojobori⁴, G. Tamiya^{1,2}.* 1) Integrated Database Group, Japan Biological Information R, Koto-ku, Japan; 2) Tokai University, School of Medicine, Isehara, Japan; 3) Chugai Pharmaceutical Co., Ltd, Gotemba, Japan; 4) National Institute of Genetics, Mishima, Japan.

The aim of this study was to identify novel polymorphic microsatellite markers throughout human genome. Our previous data indicated that microsatellite markers showed a linkage disequilibrium with disease-related alleles spanning from 100 to 200 kilobases (kb). Based on this evidence, we made a plan to identify a total of 30,000 polymorphic markers throughout human genome with 100 kb intervals. In order to investigate the microsatellite polymorphism, we performed genotyping using pooled DNA from 100 healthy Japanese individuals. 31,135 markers were identified as a polymorphic in Japanese pool. Furthermore, we investigated the characteristics of these polymorphic markers, including heterozygosity, allele number, and length of repeated unit. Mean heterozygosity and allele number were approximately 0.6 and 6.0 respectively. These data strongly suggest that we did obtain highly informative genetic markers. Next, we have mapped newly identified polymorphic markers into chromosomes, composed of the assembled genomic sequences from the repository of International Human Genome Sequencing Consortium. With these mapping data, we plan to submit the our novel the polymorphic markers to the public genomic repository. A total of 31,135 markers will aid the construction of a useful marker map of the human genome and will be a powerful tool for fine mapping of disease-susceptibility genes in genome-wide association studies. In addition, they should be applied for other purposes such as population studies.

Chromosomal regions containing high-density and ambiguous-mapped putative single nucleotide polymorphisms (SNPs) correlate with segmental duplications in the human genome. X. Estivill^{1,2}, J. Cheung², M.A. Pujana¹, K. Nakabayashi², S.W. Scherer², L-T. Tsui². 1) Genes and Disease Program, Genomic Regulation Center, Barcelona, Catalonia, Spain; 2) Program in Genetics & Genomic Biology, Research Institute, The Hospital for Sick Children, and Department of Molecular and Medical Genetics, University of Toronto. Toronto M5G 1X8, Canada.

We have explored the NCBI single nucleotide polymorphisms (SNPs) database for a correlation between the density of putative SNPs, as well as SNPs that map to different chromosomal locations (ambiguously-mapped SNPs), and segmental duplications of DNA in chromosome regions involved in genomic disorders. A high density of SNPs (14.4 and 12.4 SNPs per kb) was detected in the low copy repeats (LCRs) responsible for chromosome 17p12 duplications and deletions that cause peripheral neuropathies. None of the SNPs at the PMP22 gene were ambiguously mapped, but 93% of the SNPs at LCRs mapped on both LCR copies, indicating that they are in fact variants in paralogous sequences. Similarly, a high SNP-density was found in the LCR regions flanking the neurofibromatosis type 1 (NF1) gene, with 80% of SNPs mapping on both LCR copies. A high density of SNPs was found within LCR sequences involved in the deletions that mediate contiguous gene syndromes on chromosomes 7q11, 15q11-q13 and 22q11. We have analyzed the whole sequence of chromosome 22, which contains 14% of ambiguously-mapped SNPs, and have found a good correlation between these SNPs and segmental duplications detected by BLAST analysis. We have identified several segments of ambiguously mapped SNPs, four corresponding to LCRs involved in the chromosome 22q11 microdeletion syndromes. Our data indicate that most SNPs in LCR segments are in fact paralogous sequence variants (PSVs), and suggest that a significant proportion of the SNPs in the NCBI database correspond to PSVs within segmental duplications of the human genome sequence. These PSVs should be useful, in conjunction with BLAST approaches, to further characterize segmental duplications in the human genome. PSVs could be useful in tracing variability in copy number at these loci and in studying their potential link to human genetic disease.

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Verification of the efficacy of BAC restriction fingerprint physical maps for sequencing projects using the *Salmonella typhimurium* genome. *D. Layman, M. Sekhon, J. McPherson.* Genetics , Washington University - GSC, St. Louis, MO.

The Mapping Group at the Genome Sequencing Center provides high quality whole genome physical maps using BAC restriction fragment fingerprints. These fingerprint maps have been used to derive the minimal tiling path that represents the genomic sequence for a number of organisms, including *C. elegans*, *C. briggsae*, *Arabidopsis thaliana*, Mouse, Human, and *Salmonella typhimurium*. The construction of the physical map of the 4.8 Mb genome of *S. typhimurium* provided a unique opportunity to verify the accuracy of the BAC fingerprint maps. Most physical maps precede genome sequencing; however the construction of the *S. typhimurium* BAC map began after the sequence was finished so that a tiling path of BAC clones would be available to the scientific community for research. This tiling path was verified by comparison of BAC end-sequence to the finished genomic sequence. Once the BAC fingerprints were generated and assembled into a physical map of ordered clones the quality of the map was analyzed by comparison to the finished sequence. The completed genome sequence was divided into overlapping segments to mimic the BAC clones. Each segment was then divided into its corresponding restriction fragments to generate an in silico fingerprint. All of the in silico fingerprints were successfully incorporated into the physical map using the same software that was used for the initial physical map assembly. The ordered in silico fingerprints regenerate the finished genomic sequence indicating the accuracy of the BAC clone map.

Physical map and sequence analysis in the mouse t-complex inversion 2 region and comparison to the human syntenic region. *P. Waeltz, M. Brathwaite, M. Schroeder, D. Schlessinger, R. Nagaraja.* Laboratory of Genetics, NIA/NIH, Baltimore, MD.

The t-complex in the proximal half of mouse chromosome 17 has unusual features, including loci for many recessive embryonic lethal mutations and segregation distortion. It has been demonstrated that 20-30% of wild mice carry the t-haplotype, containing four large inversions when compared to laboratory mouse strains. In addition, there are many small duplications and deletions in the region. During the mapping of collections of cDNAs from the preimplantation and periimplantation stages, it was shown that many ESTs cluster within the t-complex region. A systematic functional study of all the genes responsible for these phenotypes and a detailed characterization of inversions and duplications require they be identified, mapped and characterized to define their biological role. Towards this goal, we have undertaken an effort to build a STS/BAC based physical map of the mouse t-complex, corresponding to a 20 cM region in an estimated 30 Mb physical distance. The current map contains 250 BACs and 570 STSs, which covers about 12 Mb of the region's total span. Contigs range in size from 300 kb to 5 Mb. The map is supported by STSs designed from BAC end sequences and the fingerprinting data.

Regarding the region of concentration to date, the physical map covers ~ 8Mb, between Brachyury (proximal end) and D17Leh55 (distal end) with one gap. Via targeted sequencing of selected clones from a minimal tiling path in this region, it is apparent that in the human syntenic region on chromosome 6, the gene content and its order is well conserved between Brachyury and Plg and the chromosomal segment has undergone an inversion during evolution between Plg and Sod2. Ongoing sequencing efforts and analysis will catalog the region's complete gene content and are also expected to a) define the inversion 2 breakpoint in greater detail and b) identify candidate gene(s) for T-associated sex-reversal, which maps here.

A Modular Approach to High-Throughput SNP Genotyping. *S. Duan¹, E. Lovins¹, R. Donaldson¹, R. Miller¹, P. Taillon-Miller¹, P. Kwok².* 1) Dermatology, Washington Univ., St. Louis, MO; 2) Cardiovascular Res. Ins. and Dermatology, Univ. of California, San Francisco, CA.

To construct high-density haplotype maps of the human genome based on single nucleotide polymorphism (SNP) markers and to perform whole genome genetic association studies of common diseases, millions of genotypes must be obtained over 2 to 3 years. In these applications, thousands of SNPs are genotyped on a few hundred samples. We report here a flexible, modular approach to SNP genotyping that emphasize low start-up instrumentation and assay development costs. We have previously described a primer extension genotyping method called the FP-TDI (template-directed dye-terminator incorporation with fluorescence polarization) assay. We have designed PCR and primer extension primers for over 1.4 million human SNPs found in public databases. The only assay development cost is therefore the 3 unlabeled PCR-grade primers for each assay. Using the 384-well reaction format, we have established procedures to obtain 9,216 to 18,432 genotypes per day, depending on the liquid handling equipment and number of workers utilized. For example, with 2 teams of 2 workers, PCR machines with twelve 384-well heating blocks, one low-end liquid handling workstation, and one FP plate reader, 9,216 genotypes (24 X 384 reactions) can be obtained per 8-hour working day. Assuming 250 working days a year, >2.4 million genotypes can be obtained with this procedure. If a high-end liquid handling robotic workstation is used in conjunction with PCR machines with 24 384-well heating blocks and the same fluorescence polarization plate reader, 3.5 workers can obtain 18,432 genotypes (48 X 384 reactions) per day. The yearly genotyping rate is therefore >4.6 million genotypes. Because the reaction protocol is extremely simple, new workers can be trained in days. Establishing new modules is therefore an easy matter. We have implemented this modular approach at two different sites with minimal effort. The procedure we report here is easily transferable and can be adopted by any group with minimal start-up cost or training. It will be able to meet the needs of all study designs that require high throughput genotyping.

Quantitative prehybridization quality assessment using three-color cDNA microarrays. *M. Hessner*^{1,2}, *X. Wang*¹, *K. Vaughan*², *L. Meyer*¹, *S. Khan*², *J. Tackes*², *M. Schlicht*³, *M. Datta*³, *H. Jacob*², *S. Ghosh*¹. 1) Department of Pediatrics, Medical College Wisconsin, Milwaukee, WI; 2) The Human and Molecular Genetics Center, Medical College Wisconsin, Milwaukee, WI; 3) Department of Pathology, Medical College Wisconsin, Milwaukee, WI.

DNA microarrays have great potential to generate new insights into human disease, however, construction methodologies still lack the ability to determine array quality and integrity prior to hybridization. We have solved this problem through printing fluorescein-labeled cDNA probes, which are compatible with target labeling dyes (Cy3 and Cy5) when using confocal laser scanners possessing narrow bandwidths. High-density arrays were evaluated using a version of Matarray [Wang et al., 2001, NAR 29, E75] modified to measure multiple prehybridization array quality parameters including correct probe address through the mapping of unique plate-specific negative controls. The average fluorescein signal/slide (1500 RFU to >15000 RFU) and the overall slide signal to noise values (calculated signal/signal+noise; 0.65 to 0.95, mean=0.92+/-0.03) varied considerably between processed slides. Comparison of hybridization results from arrays with high DNA/element and low background to those with low DNA/element and/or high background values (n=50 replicate pairs) revealed a direct and significant relationship ($R^2=0.80$, $p<0.001$) between prehybridization fluorescein image quality and replicate consistency, illustrating that microarray data quality can be improved through quality-matched prehybridization slide selection. Currently we are selecting arrays with signal to noise values >0.85, average element fluorescein intensity >3,000, and CV (coefficient of variation) of element fluorescein intensity <10%. Our approach, separates slide coating, printing, and processing from hybridization and provides a direct method for probe amplification control and plate tracking, direct examination of array/element morphology including identification of elements not printed, determination of post-process probe retention, and a means of quantitative prehybridization array quality control and selection that results in improved data quality.

Comparison of data concordance between cDNA and Affymetrix oligonucleotide microarrays. *X. Wang¹, N. Jiang², K. Vaughan³, L. Meyer¹, H. Khoo¹, S. Khan³, S. Ghosh¹, P. Tonellato², H. Jacob³, M. Hessner¹.* 1) Department of Pediatrics, Medical College of Wisconsin, Milwaukee, WI; 2) Bioinformatics Research Center, Medical College of Wisconsin, Milwaukee, WI; 3) The Human and Molecular Genetics Center, Medical College of Wisconsin, Milwaukee, WI.

The cDNA array offers design flexibility and independence of sequence knowledge as attributes. However, clone handling, clone authentication, and possible cross hybridization between related genes and splice variants are potential caveats. Alternatively, the Affymetrix oligonucleotide array offers probe sequence control, multiple interrogations per gene, and high density, while its downsides are cost, lack of design flexibility, and potentially uneven element performance. We compared the concordance of expression data between these two platforms, utilizing human (9,200 clones) and rat (18,400 clones) cDNA arrays as well as Affymetrix U95Av2 human and U34A rat arrays. The correlation and concordance between the U95Av2 and human cDNA arrays were investigated through hybridizations of UACC903 and Jurkat RNA. 4733 genes were common to both human platforms, and the correlation coefficient between detected ratios was >0.80 ; when carrying the analysis through to lists of genes differentially expressed more than 2-fold, the concordance was found to be $\sim 65\%$. Furthermore, many genes have multiple representations on each human or rat cDNA array, targeting different regions of the corresponding full-length cDNA. Among probes common between the two human platforms, nearly 21% ($\sim 1,000$) had multiple representations on either array. This enabled a probe location (5' to 3') comparison of the two systems, and an evaluation of how this parameter impacted overall data concordance. Finally, since the Affymetrix analysis involves RNA amplification, we also conducted a three-way comparison between Affymetrix, and cDNA arrays using amplified and directly labeled targets. An understanding of the characteristics of both platforms allows investigators to capitalize on the advantages that each system has to offer.

Program Nr: 1373 from 2002 ASHG Annual Meeting

Quantitative analysis of methylation status at CpG islands using Pyrosequencing™ technology. *S. Eriksson, A. Alderborn, M. Pettersson.* Pyrosequencing AB, Vallongatan 1, SE-752 28 Uppsala, Sweden.

Methylation of cytosines at CpG dinucleotides is an epigenetic modification of DNA that has profound effects in the mammalian genome. The methylated status of CpG islands present in the promoter region of a gene has a significant influence on its expression. DNA methylation has been shown to play important roles during embryonic development and X chromosome inactivation. In addition, tumorigenesis is characterized by aberrant methylation patterns resulting in, for example, silenced expression of tumor suppressor genes.

Methods for analysis of methylation patterns usually rely on bisulfite treatment of denatured DNA which converts non-methylated cytosines to uracils. Since methylated cytosines are resistant to this treatment, differentially methylated CpGs can, by this procedure, be analyzed as artificial C/T single nucleotide polymorphisms (SNP). Pyrosequencing™ technology is a rapid and accurate real-time sequencing method for analysis of short to medium length DNA sequences. Multiplex genotyping of SNP can be accurately performed and the allele frequencies of several SNPs in a pooled sample correctly quantified. We show that Pyrosequencing technology is a fast and reliable method for the detection and quantification of methylation differences at multiple specific CpG sites.

Multiplex Detection of 10 SNPs Located in the Coding Region of the Mitochondrial Genome. *J.M. Butler¹, M.D. Coble², I. Letmanyi², T.J. Parsons², P.M. Vallone¹.* 1) Biotechnology Div, Natl Inst Standards/Technology, Gaithersburg, MD; 2) Armed Forces DNA Identification Laboratory, Rockville, MD.

Single nucleotide polymorphisms (SNPs) represent an important class of DNA variation in which sequence differences between individuals are examined. A number of different SNP detection and typing technologies exist. This work focuses on the use of a SNP typing approach that works on a multi-color fluorescence capillary electrophoresis platform. Fluorescent SNP detection is accomplished (through a mini-sequencing assay) by using the commercially available SNaPshot multiplex kit.

Mitochondrial DNA (mtDNA), which is maternally inherited, can play an important role in many aspects of human identity testing due to the fact that it is more resilient to environmental degradation. A desire to gain more information than can be provided by the hypervariable regions of the mtDNA control region has led to a search for informative sites outside the control region around the remaining 15,000 base pairs of the mtDNA genome. A set of 10 highly informative sites from around the mtDNA genome has been combined into a multiplex PCR and SNP detection assay that can be detected in high-throughput fashion using multi-color fluorescence and multi-capillary instrumentation.

Experimental conditions for the multiplex amplification of nine regions in the mitochondrial genome containing ten SNP sites have been optimized. Using the multiplex generated PCR amplicons as templates ten different SNP sites are probed simultaneously in the same tube using tailed extension primers and reagents contained the ABI SNaPshot multiplex SNP kit. The products of the fluorescently labeled primer extension reactions are separated and detected on the ABI 3100 16 capillary electrophoresis instrument. The design of the multiplex PCR and extension primers as well as data interpretation will be discussed.

Genome-wide molecular and computer-based evaluation of mitochondrial pseudogenes in human nuclear DNA.

J.R. Lupski^{1,2,3}, P. Stankiewicz¹, P.E. Chen⁴, C. Kashuk⁴, M. Withers¹, A. Chakravarti⁴, N. Katsanis⁴. 1) Department of Molecular & Human Genetics; 2) Department of Pediatrics, Baylor College of Medicine; 3) Texas Children Hospital, Houston, TX; 4) Institute of Genetic Medicine and Wilmer Eye Institute, Johns Hopkins University, Baltimore, MD.

The human genome contains various higher-order structures that are often present in multiple copies and can serve as template for variation and evolution. The mitochondrial genome (mt) can be considered such a structure, since recent computational analyses of human genome sequence have predicted the presence of as many as 600 fragments of mtDNA in the nuclear genome. We have evaluated the presence, position and composition of mtDNA in nuclear DNA (*numts*) by computational analysis of four assemblies of the human genome (public and private) and by in vitro studies on nuclear DNA from several primate species. We report the mapping of 142 and 996 *numts* in mouse and human respectively, which we have validated by performing FISH with mtDNA as a molecular probe and interphase and metaphase nuclei of mtDNA depleted cells as target DNA, and by amplifying junction fragments of mtDNA insertions from human-rodent monochromosomal hybrids. In contrast to current belief, our detailed evaluation of the position and composition of each insertion suggests that clusters of seemingly independent *numts* are single mt insertions, followed by non-random divergence, which creates the "pseudoappearance" of multiple independent *numts*. Our data also indicate that a significant percentage of *numts* is transcriptionally active, as demonstrated by analysis of the presence and distribution of *numts* in dbEST and by RT-PCR on various adult and fetal tissues. Intriguingly, as much as 12% of all *numts* are intragenic, suggesting a non-random distribution in the genome. Furthermore, the sites of increased transcriptional activity correlate with positions of increased mt sequence conservation, suggesting that some portions of the mtDNA may preferentially provide material of potential functionality in nuclear DNA, although the mechanism and role of this material will require further study.

Mapping genes for human mitochondrial ribosomal proteins. *T.W. O'Brien¹, H.-R. Graack², N. Fischel-Ghodsian^{3,6}, E.B. Mougey⁴, B. Wittmann-Liebold⁵, J.E. Sylvester⁴.* 1) Biochemistry and Mol Bio, University of Florida, Gainesville, FL; 2) MediTarget GmbH, Breddinerweg 5 D-13591 Berlin, Germany; 3) Cedars-Sinai Medical Center, Los Angeles, CA; 4) Nemours Children's Clinic, Jacksonville, FL; 5) Max-Delbruck-Center for Molecular Medicine, D-13122 Berlin-Buch, Germany; 6) UCLA, Los Angeles, CA.

Mitochondrial DNA encodes tRNAs and rRNAs, but the other genes for the mitochondrial translation system are found in nuclear DNA. Upward of 85 mammalian mitochondrial ribosomal proteins (MRPs) are imported into mitochondria where they assemble into ribosomes that are responsible for translating the 13 mRNAs for essential proteins of the oxidative phosphorylation system. Since mutations in mitochondrial tRNA and rRNA can cause various pathological states, we hypothesize that mutations in MRP genes are also candidates for human disorders. Our approach is to use N-terminal and internal amino acid sequence data obtained from purified bovine MRPs (O'Brien, et al. (2000) *J Biol Chem.* 275: 18153) to search EST databases. Chromosome map positions for the MRP genes are ascertained by in situ hybridization (FISH) with genomic sequences and/or by using in silico methods to search Genbank and GeneMap through NCBI. At present, we have over 78 different human MRPs at various stages of characterization. We are currently investigating one MRP as a potential candidate for Russell-Silver Syndrome (RSS), a dwarfism characterized by low birth weight and lateral asymmetry, characteristics that are consistent with reduced mitochondrial function. DNA from RSS patients is being analyzed for possible mutations in MRPs. In addition to studying their clinical relevance, long term characterization of MRP genes should lead to important insights into mammalian evolution, coordinate regulation of nuclear and mitochondrial gene expression, and ribosome function. This work is being done as part of the Mammalian Mitochondrial Ribosomal Consortium and is supported by NIH/NIDCD grant RO1DC04092 and the Nemours Research Program.

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High Throughput Automated Mutation Discovery. *P.S. Andersen¹, C. Jespersgaard¹, C. Brown², J. Vuust¹, M. Christiansen¹, L.A. Larsen³.* 1) Dept Clinical Biochemistry, Statens Serum Inst, Copenhagen, Denmark; 2) Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404; 3) Wilhelm Johannsen Institute of Medical Biochemistry and Genetics, University of Copenhagen, Denmark.

Screening for unknown mutations has been dependent on several methods, all of which have a relatively high sensitivity. In the present study we describe a mutation discovery procedure that is applicable when screening a large number of samples for unknown mutations where we use automated capillary array electrophoresis single strand conformation polymorphism (CAE-SSCP). Using DNA sequencing as the golden standard we validate the method by using a number of in vitro constructed mutations. We have determined the sensitivity and specificity of the system and shown the high throughput capacity of the screening procedure.

Program Nr: 1378 from 2002 ASHG Annual Meeting

Full multiplexing of up to 48 SNP genotypes on the SNPstream® UHT Genotyping System. *M.A. Kunkel, J. Baisch, C. Barceleau, C-Y. Huang, C. Gelfand.* Orchid BioSciences, Inc, 303A College Road East, Princeton, NJ 08540.

Wide-ranging commercial applications for SNP genotyping are enabled by the accuracy, flexibility and cost-effective platform scalability of SNP-IT™, the core genotyping technology of Orchid BioSciences. Adaptation of this single biochemistry to a variety of novel microarray platforms, known as SNP-IT *tag-array*, provides the necessary flexibility to enable studies ranging from research bench-top applications to the highest industrial throughput needs. Ultimately, the utility of genetic variability in pharmacogenetic and pharmacogenomic studies is dependent on flexible, cost effective genotyping systems.

The flexibility of any platform is directly related to its ability to scale to the users requirements for a particular study. Higher levels of multiplexing allow for both ease of use in experimental design and reductions in overall reagent and sample costs. The SNPstream UHT Genotyping System, currently used routinely at the 12-plex level, is being expanded to 48-plex reactions. Higher multiplexing is of particular utility for human parentage/forensic testing, where 48 genotypes can provide sufficient confidence in the resultant genetic profile to distinguish one individual from another. A single individual paternity result can therefore be collected utilizing only one well of the assay plate. As well, SNPs for mapping studies can be batched into moderate-sized SNP panels, to quickly identify areas of association. By automating the process, throughputs of vast proportions, potentially in excess of several million genotypes per day, can be achieved on a single instrument. With the assays and data formats standardized, small and large cross-study analyses are intrinsically compatible. Application of this higher multiplexing with the SNPstream UHT Genotyping System will be demonstrated, featuring paternity and other identity studies.

Digital Oligonucleotide Tiling (DOT): A novel genome scanning assay. *A.P. Shuber, K. Boynton, J. Skoletsky.*
EXACT Sciences Corp, Maynard, MA.

Background: Genomic scanning methods such as restriction fragment analysis, allele specific oligonucleotide hybridization chips, and single nucleotide primer extension can be powerful assays for the detection of mutations. One drawback of using these methods is that the mutations must be known and primers specific to these mutations must be utilized. We have developed a simple and efficient assay capable of scanning for unknown point mutations. We report the use of this assay in the detection of single base mutations in a model APC polymorphism system, as well in colonic tissues from colorectal cancer (CRC) patients. **Methods:** The DOT assay was developed with a model system comprised of a 221 bp target region consisting of a polymorphism (A/G) at codon 1493 in the Mutation Cluster Region (MCR) of the APC gene. Scanning was performed by hybridization with an array of 17 bp oligonucleotides, in solution, to a sequence of APC MCR DNA amplified from PBMCs. After hybridization, an S1 nuclease cleavage was performed and target template DNA that had not been fully hybridized to the wild type oligonucleotide array due to mismatches was cleaved. The presence of a mutation was determined by the detection of strand truncation. Additionally, this assay was performed on DNA from colonic biopsies in CRC patients. **Results:** When template DNA containing a mismatch was hybridized to the wild type oligonucleotide array, cleavage was detected. When template DNA without a mismatch was assayed, no cleavage product was detected. When the DOT assay was used on DNA from microdissected tissue mutations were detected and confirmed by standard DNA sequencing methods. **Conclusions:** We have developed an in-solution Digital Oligonucleotide Tiling (DOT) genomic scanning assay that is a simple and efficient method of screening for mutations. Additionally, we have demonstrated that this assay is capable of mutation detection in DNA purified from clinical samples (CRC tissues). This novel method should be a valuable addition to other methodologies that scan for unknown mutations.

Four-color multiplex PCR assay for the simultaneous detection of four allelic variants in a closed tube using a single thermal cycler program. *L.A. Ugozzoli, D. Chinn, K. Hamby.* Life Science Group, Bio-Rad Laboratories, Hercules, CA.

The 5' nuclease assay is one of the most widely used allele-specific homogeneous assays. The assay takes advantage of the 5'→3' nuclease activity of Taq DNA polymerase to cleave fluorescently labeled allele-specific oligonucleotide (ASO) probes when they hybridize to PCR products during the PCR annealing phase. We developed a real-time multiplex assay for the simultaneous detection of up to four allelic variants in one closed tube. The assay combines the power of multiplex PCR with the specificity provided by ASO hybridization using the 5' nuclease assay format. We applied the four-color assay for the simultaneous detection of the factor V Leiden (FVL) G1691A and prothrombin (PT) G20210A mutations, the two most common known genetic risk factors for venous thrombosis in Caucasians. Human genomic DNA is prepared from whole blood using standard procedures. A 97 bp DNA sequence of the coagulation factor V gene is co-amplified with a 111 base pair DNA sequence of the coagulation factor II (prothrombin) gene using four PCR primers. In addition, the PCR reactions included four differentially labeled ASO probes for the specific detection of the different FVL/PT G20210A genotypes. To evaluate the assays performance characteristics, we performed a method-comparison study. Results generated with the four-color assay were compared with those obtained with a reference method based on the use of PCR and restriction enzymes. We analyzed 52 DNA samples with known FVL / PT G20210A genotypes that were previously genotyped with the reference method. We found a 100% correlation between the results generated by both methodologies. We conclude that the four-color assay is specific and reproducible for the detection of the FVL/PT G20210A mutations, and it can be easily adapted for the detection of other SNPs. The assay, which will be useful for both molecular diagnostic and research laboratories, offers numerous advantages over more traditional methods for the detection of the FVL and PT G20210A mutations. The advantages include speed and simplicity of the method, reduced labor, reduce risk of cross-contamination, and higher throughput.

Application of a DNA microarray-based comparative genomic hybridization (Array-CGH) method to the study on the genetic effects of Atomic bomb radiation; Report 2. *N. Takahashi¹, K. Sasaki¹, N. Tsuyama¹, M. Kodaira¹, K. Sugita², H. Katayama², K. Hiyama³, E. Hiyama³.* 1) Lab Biochem Genetics/Dept Gen, RERF, Hiroshima, Japan; 2) Dept Info Tech, RERF, Hiroshima, Japan; 3) Sch Med Hiroshima Univ, Hiroshima, Japan.

For over 50 years, the Foundation has conducted a study on the effects of A-bomb radiation on human germ cells. For the purpose of conducting the study at the genome-level, it is essential to efficiently collect a large volume of genetic information from a sufficient number of samples. It is also important to accurately detect a deletion which occurs on the gene on autosome (in most cases, radiation-induced mutations appear to be about 1 Mb deletion), since the deletion appears as a change of 2 copies of alleles to one. An Array CGH can detect single-copy decrease and increase from normal diploidy. For this, the Array CGH is considered to be an effective method for our study. Last this meeting, we reported that trial experiments using about twenty clones had proven this method could reliably detect deletion-type mutations. Here, we examined the feasibility of this method by using the arrays having numerous Bac- or Cosmid-clones, and also developed a computer-based speedy data processing method. We constructed arrays to which 676 cloned DNAs have been immobilized. Genomic DNA from cell lines established from children of A-bomb survivors or myeloma patients were used as test samples. In first stage, DNA to be used as an internal control was labeled with Cy5, and each test DNA was labeled with Cy3. After they were mixed, they were hybridized and quantified. By using software developed by our foundation, we determined the presence and absence of abnormality from the fluorescence intensity ratio of Cy5 to Cy3. Next stage, conversely, mixture of Cy3-labeled control and Cy5-labeled test samples were used as probes. We identified putative polymorphic variations in some spots. These results indicate that the array CGH, together with computer-based data analysis, can detect changes in the copy number of specific DNA fragments speedily and efficiently. Thus, this means that this method is one of the most useful for our study.

A Survey of Sequence Divergence of Genes on Human Chromosome 21 between Human and Other Primates Reveals Variation of Evolution Rhythms among Transcription Units. *J. Shi¹, H. Xi^{1,2}, Y. Wang¹, C. Zhang¹, L. Jin¹, Y. Shen¹, L. Jin^{1,2}, W. Huang¹.* 1) Chinese National Human Genome, Shanghai, China; 2) The Institute of Genetics, Fudan University, Shanghai, China.

The study of genomic divergence between human and primates, our living relatives will shed lights on the origins of human being, unique human traits and even the genetic basis for some human diseases. Chromosome 21 is the smallest chromosome in human genome and has important regions related to mental retardation and other diseases. In this study, we sequenced the coding and regulatory regions of 127 known genes on human chromosome 21 in DNA samples from human and chimpanzees, and a part of the corresponding genes from orangutan, gorilla and macaque. We identified 3,003 nucleotide variations between Human and Chimpanzee over a total of 399,264bp sequences. The difference of coding, promoter and coding boundary regions were 0.51%, 0.88% and 0.85% respectively, much lower than the previously reported 1.23% of randomly sequenced genomic regions. Significant variations in divergence between human and chimpanzee were observed among the genes. An Ka/Ks analysis allows identification of genes and their segments that possibly went through positive Darwinian selection. Furthermore, the variations that are specific to human lineage might lead to the functional evolution and attribute to the humanness phenotype through changing structure and/or dosage of the proteins expressed. A phylogenetic analysis unambiguously showed that chimpanzees were our closest relatives to the exclusion of other primates.

Pyrosequencing™ assess the most common Cystic Fibrosis mutations. *S.I. Toth¹, A.L. Schiller², J. Dunker², U. Larsson², M. Storgards², A. Alderborn².* 1) Pyrosequencing AB, Westborough, MA; 2) Pyrosequencing AB, Uppsala, Sweden.

Cystic Fibrosis is a lethal recessive genetic disorder in Northern European populations affecting 1 live birth in 1600-2500. In contrast to many other recessive disorders, the carriers of CF mutations (1 in 25 Caucasians) have no biochemical or physiological alterations by which they could readily be identified. As a consequence the search for genetic markers became a matter of decisive importance. Thirteen years after the discovery of the cystic fibrosis transmembrane conductance regulator (CFTR) gene and the characterization of over 1000 mutations, CF is now to become the first disease targeted for population-wide genetic screening.

Pyrosequencing AB (Sweden) offers several genotyping systems well suited to meet the demand for research and routine testing of mutations in the CFTR gene, providing simple, rapid, cost efficient and accurate detection of relevant mutations. Pyrosequencing is a DNA sequencing technology based on real-time detection of pyrophosphate released upon nucleotide incorporation. Highly reliable CF assays have been designed and developed for 61 of the most common CFTR mutations, including point mutations as well as insertions and deletions. The assays are greatly condensed including multiplex design of both PCR and sequencing reactions. The assays have the potential to easily include less common mutations in the same analysis simply by extending the number of nucleotide dispensations in each pyrosequencing reaction. In addition, the same assays have been used for population-based determination of carrier frequencies using allele quantification.

Transcriptional response to ionizing radiation exposure. *K. Jen, V.G. Cheung.* Department of Pediatrics, University of Pennsylvania, Philadelphia, PA.

Medically, ionizing radiation (IR) is commonly used for diagnostic and treatment protocols. However, IR poses a major threat to healthy cells by compromising genomic integrity and cellular viability. To identify genes involved in the cellular response to IR stress, we used oligonucleotide microarrays to examine the transcriptional changes that occur following IR exposure.

We irradiated lymphoblastoid cells at a dose of 3Gy or 10Gy and tracked the mRNA levels of approximately 12,000 genes at 1hr, 2hr, 6hr, 12hr, and 24hr after IR exposure. An unirradiated sample was prepared to assess baseline gene expression levels. We determined expression changes by comparing irradiated samples to the unirradiated control. Among the genes surveyed, 329 and 864 genes changed transcript levels in response to 3Gy and 10Gy of IR, respectively. Genes that showed the largest expression changes include *ATF3*, *GADD45A*, *PPM1D*, *CDKN1A*, and *FDXR*. As expected, a large portion of the characterized genes (22%) are involved in pathways associated with IR stress response including DNA damage repair, cell cycle regulation, cell proliferation, and/or cell death. We also identified many genes not known previously to be involved in the IR-response.

We describe two classes of expression changes following IR stress: (1) rapid early expression changes and (2) gradual expression changes at later time-points (6 hr post-irradiation and beyond). We denote these IR-responsive genes as early or late response genes. Different genes associated with cell cycle regulation and apoptosis are involved in these two phases of the IR response. For example, *TNFRSF6* (Fas/Apo-1) and *TNFRSF10B* (DR5) show early expression changes while *BAX* changes expression levels at later time-points. Our data also indicate dose-dependent effects on temporal patterns of gene expression. At a lower IR dose, most early response genes return to basal levels shortly after induction. However, at a higher dose, early response genes exhibit more rapid gene expression changes that are greater in magnitude and last longer in duration. In this presentation, we will describe results from our genomic approach in studying the process of cellular adaptation to IR stress.

A single-tube multiplex minisequencing assay for simultaneous genotyping of 6 single nucleotide polymorphisms (SNPs) of the MDR1 gene. *C.G.L. Lee^{1,2,4}, P.C. Gwee¹, K. Tang¹, J. Chua¹, S.S. Chong^{3,5}.* 1) Div Medical Sci, Natl Cancer Ctr, SINGAPORE; 2) Biochemistry; 3) Paediatrics, Natl Univ Singapore, SINGAPORE; 4) Medicine; 5) Paediatrics, Johns Hopkins School of Medicine, Baltimore, MD.

Responses to different drugs can vary widely between different individuals as a result of genetic variations in drug metabolizing enzymes, transporters, receptors or other co-factors. The MDR1 multidrug transporter has been shown to efflux a wide variety of structurally and functionally unrelated drugs. Genetic polymorphisms within the MDR1 gene have recently been intensively studied, and the C3435T single nucleotide polymorphism (SNP) has been associated with differences in drug response. However, this SNP, a silent polymorphism, was found to be contradictorily associated with different changes in MDR1 phenotype. We recently demonstrated through haplotype analyses that this SNP is in linkage disequilibrium (LD) with two other SNPs within the MDR1 gene. We hypothesized that strong LD between C3435T and different unobserved causal SNPs in different study populations may explain the association of the 3435T allele with different MDR1 functional changes. To simplify the genotyping process, we have developed a rapid single-tube minisequencing assay that simultaneously genotype 6 common SNPs spanning approximately 80 kb of the gene. In this assay, a multiplex PCR amplification of 5 different fragments containing the 6 MDR1 SNPs is performed. Excess primers and unincorporated nucleotides are enzymatically degraded prior to addition of SNP-specific minisequencing primers which contain non-specific tails of differing lengths. Single nucleotide primer extension was performed using the SNaPshotTM kit. Unincorporated fluorescent ddNTPs were enzymatically inactivated and SNP-specific primers with fluorescently incorporated ddNTPs were resolved by capillary electrophoresis on the ABI 3100 and analyzed using Genotyper 3.7TM. The development of this rapid and robust assay will greatly simplify multiple MDR1 SNP genotyping and subsequent haplotype construction, thus facilitating more efficient identification of the causal SNPs in linkage disequilibrium with C3435T in the different study populations.

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Miniaturization and automation of SNP genotyping using AlphaScreen. *J.-A. St-Pierre, J. Lian, D. Rivard, E. Robitaille, D. Chan, L. Beaudet.* PerkinElmer Life Sciences, 1744 William, Montréal, QC, Canada H3J 1R4.

In AlphaScreen homogeneous assays, signal is generated when a donor and an acceptor bead are brought into proximity. Laser irradiation of the donor beads generates singlet oxygen that reacts with the acceptor beads in close proximity, resulting in an amplified light signal. AlphaScreen beads, with oligonucleotides attached at their surface, can be used to perform very rapid and accurate single-nucleotide polymorphism (SNP) typing assays. In these single-well assays, based on allele-specific hybridization, AlphaScreen beads and probes are combined with the PCR reagents and added to PCR plates with the genomic DNA prior to amplification. The assay set up requires only two additions to the plates and is easily automatable. After amplification and probe hybridization, genotypes are analyzed without having to purify the PCR products or to perform additional enzymatic reactions. We will present genotyping data for several SNP assays that were miniaturized and automated using the PlateTrak liquid handling workstation (PerkinElmer Life Sciences). The accuracy of the AlphaScreen assays in 384-well format will also be evaluated.

An updated physical map encompassing the HCHOLA3 region at 1p34 and analysis of 37 functional and positional genes. *C. Boileau*^{1,3}, *M. Abifadel*^{1,2}, *L. Villegier*¹, *M. Varret*¹, *JP. Rabes*^{1,3}, *D. Allard*¹, *A. Robin*¹, *M. Devillers*¹, *M. Krempf*⁴, *C. Junien*^{1,3}. 1) INSERM U383, Hosp Necker-Enfants Malades, Paris, France; 2) Saint-Joseph University, Beirut, Lebanon; 3) Hosp Ambroise Pare, Paris, France; 4) Hosp Hotel Dieu, Nantes, France.

Autosomal dominant hypercholesterolemia (ADH) is mainly caused by mutations in the low density lipoprotein receptor gene (LDLR) or in the gene encoding its ligand: apolipoprotein B (APOB). However, we have shown that more than 30% of ADH cases are not associated with mutations in either these two loci. We reported one French pedigree with ADH in which we excluded linkage to the LDLR and APOB genes and mapped a new locus HCHOLA3, (previously named FH3) in a 9 cM region at 1p34-p32. Hunt et al. confirmed our results with the study of an Utah ADH family and localized the HCHOLA3 locus in an overlapping 16 cM region. The 1.6 cM common interval between D1S211 and D1S2130 maps at 1p34. We have constructed a 2.9 Mb BAC transcript map encompassing the candidate interval and bridged the four sequence contigs of this region available from the working draft sequencing program. We localized 26 transcripts and identified 11 new genes using Genscan exon prediction program. Several of these genes could be implicated in lipid metabolism. We have sequenced over 300 exons from 37 genes. We identified more than 90 single nucleotide polymorphisms (SNP) with 15 new non-synonymous amino acid changes. As yet, no ADH causing mutation has been found in the original family nor in 3 newly identified French pedigrees linked to this genetic interval. In conclusion, we report the continuous physical map of the FH3 region, the exclusion of 37 regional candidate genes and describe 40 SNPs newly identified. This work should facilitate identification of HCHOLA3 as well as other genes mapped at 1p34.

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Genomic Hybrid Identity Profiling (GenomeHIP) of chromosomal regions Identical By Descent. *P. Brooks, J. Hager, S. Maillard, J.-P. Saraiva, M. Vanpeene, C. Marcaillou.* IntegraGen, SA, 4 rue Pierre Fontaine, 91000 Evry, France.

We have elaborated a procedure to identify chromosomal regions that are identical by descent (IBD) between two related individuals. The approach combines and improves on some previously described concepts, in that genomic DNA from two individuals is fragmented, mixed to form hybrids and then challenged with DNA mismatch repair proteins (Nelson et al.), and IBD-enriched clones are identified by two color hybridization to arrays of marker DNA's (Snijders et al.). The novel procedure has been designed for high throughput production starting with modest amounts of DNA samples. Treatment of paired samples includes internal quality controls that enable simultaneous validation of the elimination of mismatch-bearing fragments and the preservation of perfectly paired hybrid fragments. Whole genome amplification (WGA) of the final product of the procedure produces sufficient DNA for labeling for hybridization. BAC generic amplification (BGA) is used to generate DNA for printing arrays. A novel method has been developed to assess WGA and BGA protocols for preservation of complexity and relative representation. Addition of pg amounts of lambda DNA or of varying ratios of rice BAC DNA's showed that the procedure can discriminate between one, two and five human genomic copy equivalents. We have validated the entire procedure using CEPH family DNA's and chromosome-specific BAC arrays. Clusters of clones with high fluorescent signals corresponded to regions of known IBD.

Nelson SF, McCusker JH, Sander MA, Kee Y, Modrich P, Brown PO (1993)

Nature Genet. 4:11-18.

Snijders AM, Nowak, N, et al., Gray JW, Pinkel D, Albertson DG (2001)

Nature Genet. 29: 263-264.

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Graphical tools for visualizing the Human Genome. *R.L. Gibson, R. Little.* Genome Therapeutics Corp., Waltham, MA.

There is now more sequence data available for the human genome than for any other organism. This data, which consists of a combination of genomic and derived sequence data (e.g. cDNAs and ESTs), provides invaluable resources for the investigation of the molecular basis of diseases in humans. However, the search for causes of genetic disease requires a large amount of molecular and genetic information to be presented in various displays in order to enable researchers to interpret and utilize the data. We have created 3 Perl/Tk programs to help visualize data in this pursuit: **transcript_map**, which assists in ordering genetic markers by visually displaying clones and the markers they contain; **gene_view**, which displays low-level details of a gene, showing contigs, primers, exons, and polymorphism; and **snp_view**, which provides a high-level view of a chromosome region, showing clones, SNPs, exons, markers, genes, and promoters. These applications provide the basis for viewing all of the commonly available forms of sequencing and cloning data used in these studies.

Construction of a physical map of the distal tip of human chromosome 21p. *H. Dimitropoulos*¹, *S.M. McCutcheon*¹, *Z. Burki*², *J. Doering*², *M.R. Cummings*¹. 1) Dept. of Biological Sciences, University of Illinois at Chicago, Chicago, IL; 2) Dept. of Biology, Loyola University, Chicago, IL.

The short arm/telomeric region of chromosome 21p is a model for understanding the organizational landscape, sequence and eventually, the function of a heterochromatic region of the genome. We are creating a physical map of the short arm subtelomeric region of human chromosome 21 using a combination of somatic cell hybrids, Southern blotting and FISH. This region, called the distal tip, spans the distance from the distal rDNA junction to the telomere. We are using a hybrid cell line, X;21, carrying a translocated copy of chromosome 21 as the only human component. The X;21 translocation retains only the distal tip of chromosome 21. Our data indicates that the distal tip of this copy of chromosome 21 is 1.6 Mb long and contains more than a dozen different repetitive sequence families organized in a complex fashion. Using blots from pulsed field gel electrophoresis we have established that b satellite, satellite I and satellite III are highly represented in this region. An analysis of digests using over 20 restriction enzymes shows a complex pattern of linkage relationships among b satellite, satellite III, satellite I and AK500 in this small region of the genome. As an example, we have identified a 53 kb *NcoI* fragment that contains b satellite, satellite III and AK500, further underscoring the complexity of this subtelomeric region. Additionally, at least two types of the b satellite family have been identified on the distal tip: b₂ and b₇. These linkage relationships provide a framework for the construction of a detailed physical map of this region of the short arm. Most of the repetitive sequences on the distal tip are also found in the proximal region of the short arm of chromosome 21 where they are organized into larger, more homogeneous blocks. This physical map represents the first detailed analysis of an acrocentric short arm subtelomeric region.

Characterization of the organization of the 9-kb subtelomeric repeat on human chromosome 21. S.M.

*McCutcheon*¹, *H. Dimitropoulos*¹, *E. Kalinina*¹, *J. Doering*², *M.R. Cummings*¹. 1) Dept. of Biol. Sci., University of Illinois, Chicago, IL; 2) Dept. of Biology, Loyola University, Chicago, IL.

Little is known about the organization of the repetitive sequences on the subtelomeric region of the human acrocentric p-arms. Thoraval *et al.* (PNAS 93:4442,1996) isolated a 9-kb subtelomeric repeat which was localized by FISH analysis to all acrocentric p-arms and the centromeric region of chromosomes 3 and 4. Additionally, they showed this repeat is similar to a chimpanzee telomere-associated sequence. To further characterize the 9-kb repeat, we initiated an analysis of the organization of this sequence on a single acrocentric chromosome using a deletion hybrid panel and telomere-anchored PCR analysis. Two cell lines derived from a translocation between HC 21 and HC X were critical in this analysis. One cell line contains the distal region of the 21 p-arm from the telomere to the rDNA genes, while the other cell line contains the reciprocal translocation chromosome containing HC21 sequences from the rDNA locus to 21qter and two additional chromosomes (HC 3, HC 5). Southern analysis using these and additional deletion hybrid cell lines localized the 9-kb repeat to regions both proximal and distal to the rDNA locus. Additionally, preliminary data indicates the repeat organization on HC 3 is different from the organization on HC 21. To determine the proximity of this repeat to the telomeric repeats on HC21, the same PCR strategy as Thoraval, *et al.* was employed. When using total genomic DNA, we isolated the same junction site as Thoraval *et al.*. However, PCR analysis using DNA from a hybrid cell line containing HC 21 as the only human genomic component yielded a different junction sequence. The cloned HC21 junction sequence is at a different location in the 9-kb repeat than the junction site isolated from genomic DNA. However, only a few telomeric repeats are present in this clone which may represent the junction of the 9-kb repeat with other sequences, perhaps from the proximal locus. The organization of this and other cloned junctions are currently under investigation.

Putting SNPs to the test: Retrieval rates and coverage using public SNP databases. *B. Burwinkel, U. Nentwich, S. Steinlicht, S. Bangert, S. Grant, R. Tolle.* Genetic Epidemiology, LION bioscience AG, Heidelberg, Germany.

Single nucleotide polymorphisms (SNPs) are of ever increasing importance as markers for gene discovery, target validation or as diagnostic markers for genes involved in complex diseases. Today, the vast majority of SNPs in databases are neither validated nor are frequency data available. So it is of great interest to know what verification rate can be expected from database SNPs, how many new SNPs might be expected with own SNP detection efforts and the rate of SNPs in allelic association. 33 cardiovascular disease candidate genes were analyzed with a focus on 26 nuclear receptor (NR) genes, 2 NR cofactor genes, 5 lipid metabolism related enzymes and transporter genes. An analysis of public SNP databases yielded 1278 polymorphisms for these genes. From this list 266 SNPs were chosen for further analysis according to the following criteria:

SNPs that lead to amino acid changes or might have a regulatory influence e.g. affecting splicing, transcription efficiency or mRNA stability. Uniform distribution of SNPs across the genetic locus was an additional criterion. SNPs with several submissions or SNPs with available frequency data greater than 5% were preferably chosen. We avoided choosing SNPs lying in repetitive sequences or in regions with ambiguous mapping positions. The selected SNPs were verified in 24 unrelated German individuals by PCR amplification and direct sequencing of approximately 250 bp surrounding the respective SNP. Of the 266 tested SNPs, 141 SNPs (53%) were verified. The success rate was higher when analyzing SNPs that had several submissions (66%) than for SNPs with single submitters (45%). 104 new SNPs were detected and finally 108 SNPs were screened in assays.

As just approximately a half of the annotated SNPs were reproducible and occur with a frequency above 5%, it is advisable perform a pre-selection of database SNPs and validated SNPs prior to mass screening. Despite the increasing number of SNPs in databases, there is still a remarkable success rate in finding new SNPs with in-house screening efforts.

SNP allele frequency determined for pooled samples in multiplexed assays. *C.A. Gelfand, K.E. Scott, S.A. Varde.*
Orchid BioSciences, Inc., Princeton, NJ.

Studies associating phenotype to genotype are becoming increasingly prominent in the post-genomic era. As a first step for many association studies, the analysis of so-called “pooled” samples provides a simple way to screen SNPs for any potential correlation to phenotypes, or vice versa. A pooled sample is simply an equal mixture of any desired number of individual DNA samples, which can be grouped together randomly or, often, grouped by having similar phenotypes, such as disease versus control populations, selective breeding traits, or any other quantitative or qualitative traits. When used as an input into a SNP genotyping assay, the resulting data reflects the allele frequency of the population compiled into the pool, rather than the simple heterozygote or homozygote alternatives for individual samples.

We have used SNPstream® UHT Genotyping System, one of Orchids tag-array SNP genotyping platforms, to determine allele frequency in pooled samples. Representative data will be shown comparing the allele frequency calculated from genotyping of a panel of individual SNPs to the observed frequency of the pooled sample of the same DNAs. Such experiments demonstrate the basic ability to analyze pooled samples. Further control experiments, with the input samples being mixtures of various ratios of known individual samples, demonstrate the accuracy of SNPstream UHT Genotyping System to report allele frequency. Importantly, the allele frequency data is collected in the same multiplex assays, including both PCR and SNP-IT™, that give SNPstream UHT Genotyping System its inherent flexibility and throughput.

Program Nr: 1394 from 2002 ASHG Annual Meeting

Solution for high throughput SNP validation. *D. Bishop, C. Brown, E. McIntyre, C. Frantz, Y. Lou, G. Mitra, T. Hatch.* Applied Biosystems, Foster City, CA.

Genomic variation, as identified by single nucleotide polymorphisms (SNPs), provides key information to understanding biological mechanisms and disease susceptibility. However, to effectively use this information for pharmacogenomic or association studies SNPs should be validated to determine allele frequency and the correlation between phenotype and genotype prior to completing large genotyping projects. Applied Biosystems offers a complete solution for performing high throughput SNP validation by combining various capillary electrophoresis instruments, reagents and software applications. We present here the workflow for generating over 15,000 genotypes within 24 hours using the ABI PRISM® 3100 Genetic Analyzer on a 22-cm capillary array. SNP genotypes were produced using the ABI PRISM® SNaPshot® Multiplex Kit, a single-base extension chemistry kit that utilizes a fifth-dye labeled size standard to interrogate multiple loci within a single capillary. Prior to using the SNaPshot® Multiplex Kit, the SNaPshot® Primer Focus™ Kit was used to evaluate potential SNP probes and provide key information to enable automated analysis within GeneMapper™ Software Version 3.0. This workflow provides the research community with a convenient method of completing their SNP validation studies with a high degree of accuracy in a short amount of time.

Plurex™ Multiplexing System: High-throughput production of PCR amplicons for genetic analyses. *D. Albagli, B. Chen, A. Sassi, D. Siu, R. Anderson.* Aclara BioSciences, Mountain View, CA.

Generation of the tens to thousands of amplicons needed by genetic analysis platforms (arrays, mass spec, electrophoresis) is a bottleneck in experimental designs and a high-cost step. Typically amplicon generation is done as (a) a single-plex reaction, necessitating high reagent and template consumption, or as (b) a tube multiplex reaction, requiring extensive development to ensure the primer pairs are compatible. Even if multiplexed primer sets have been developed, amplicon drop-out is not uncommon. Modifying a multiplex set also requires full validation of the new set because the effects of primer-primer interactions are poorly understood.

At ACLARA we have developed the Plurex device to overcome the bottlenecks and limitations of tube-based multiplexed PCR reactions. In the device each PCR reaction occurs in a spatially distinct region, substantially isolated from one another, and so occur as a collection of single-plex reactions.

The Plurex device is an injection-molded part that contains 96 sample channels in standard SBS plate format. A channel is a narrow spiral that has wells at each end designed for manual or robotic fluid I/O. Each channel has 10 distinct zones to which 10 different primer sets may be spontaneously attached. User-defined primer sets are first added and bound in the channel, and then all other reagents, e.g. enzyme, dNTP's and buffer, are added to the channel and shared across all the reactions. The Plurex device is then thermocycled on standard flat-block instruments.

Users can further multiplex reactions within each reaction zone of the channel. Multiple primer sets may be programmed to segregate within each zone, to enable for example a 50-plex reaction in each channel in which there are 10 different localized 5-plex reactions. In a Plurex device the design and validation of multiplex PCR is significantly simplified while yielding highly multiplexed performance, high productivity and reduced costs and sample usage.

Program Nr: 1396 from 2002 ASHG Annual Meeting

Understanding the microsatellite mutation mechanism in the human genome in silico. *Y. Lai*¹, *F. Sun*^{2,1}. 1) Department of Mathematics, University of Southern California, 1042 West 36th Place, DRB142, Los Angeles, CA 90089-1113; 2) Department of Biological Sciences, University of Southern California, 1042 West 36th Place, DRB142, Los Angeles, CA 90089-1113.

Microsatellite markers are widely used for genetic studies, but their mutation mechanism remains unclear. To investigate the relationship between the mutation rate and the number of repeat units, we collect dinucleotide microsatellites distributions of different motifs in the human genome. We consider a model that microsatellite mutations consist of point mutations, single stepwise extension slippage and contraction slippage. We explore the mutation mechanism using the equilibrium equation of a continuous time Markov process. We first study the widely used symmetric model assuming that the extension rate equals the contraction rate. We find that the total slippage rate decreases when the number of repeat units is above a threshold. This conflicts with experimental observations. We develop a novel approach to understand the mutation mechanism of microsatellites. We cluster microsatellites with similar number of repeat units into groups and assume that microsatellites within the same group have the same extension and contraction rates. We estimate the extension and contraction rates using the observed distribution. For poly-CA, we find that the contraction rate increases while extension rate slightly decreases with the number of repeat units. The results are consistent with experimental observations. This is the first study to show such phenomena based on genomic sequence data. We also estimated the extension and contraction rates for other motifs.

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Genotyping with Orchid's SNP-IT™ Technology Using Singlex and Multiplexed Tag-based Platforms. *B.C. Sundaresan, J.C. Mylet, T. Mulcahy, C. Crego.* Application Res, Orchid BioSciences Inc, Princeton, NJ.

With the increasing availability of information on DNA sequence and single nucleotide polymorphisms (SNPs), it has become increasingly important to develop effective assays for SNP scoring. As part of our Platform Propagation™ strategy, Orchid has developed several platforms with varying throughputs, harnessing the power and flexibility of SNP-IT™, Orchid's proprietary single base primer extension technology. Having a range of platforms provides not only a choice of instrumentation for the customer but also facilitates moving from one platform to another as their study scope changes, without changing the basic assay chemistry. Here we demonstrate that genotyping results obtained with the various platforms are highly concordant, supporting the platforms' flexibility and compatibility, without compromising data quality.

Loss of Heterozygosity Studied using SNPs as Markers. *S.A. Varde¹, J.A. Lathrop¹, M.A. Donaldson¹, J.M. Maris², G. Hii², P. Fortina², E. Rappaport², W.M. Ankener¹, J.F. Studebaker¹, S.V. Alfisi¹, M.S. Phillips¹, M.T. Boyce-Jacino¹, C.A. Gelfand¹.* 1) Research and Development, Orchid BioSciences, Inc., 303 College Road East, Princeton, NJ 08540; 2) The Childrens Hospital of Philadelphia, Division of Oncology, ARC902A 34th and Civic Ctr. Blvd., Philadelphia, PA 19104.

The task of elucidating the human genome sequence has led to a wide range of projects that utilize the genome sequence as a starting point, such as genotyping of single nucleotide polymorphisms (SNPs). Orchid BioSciences, Inc. has developed a new generation of SNP genotyping technology based on SNP-IT tag array, a next generation technology that combines multiplexed PCR and Orchid's proprietary SNP-IT single-base extension genotyping with a generic tag-array readout. Multiplexed SNP-IT reactions are spatially sorted into singlex for analysis by hybridization to the tag arrays. Among the advantages of SNP-IT tag array is its generic nature, allowing the same single array design to be used to genotype any SNP of interest. Representative SNP genotyping using this tag-array approach will be presented.

The flexibility of the SNP-IT tag-array allows SNPs to become straightforward tools for a wide range of genetic studies. Our collaborators at The Childrens Hospital of Philadelphia are conducting loss of heterozygosity (LOH) studies on human-derived neuroblastoma cell lines and primary tumor samples. We will present SNP genotyping data showing LOH chromosome band 1p36 in cell lines and clinical samples. Forty-one SNPs were selected within 1p36.3, and also sixty SNPs at 16p12-13 served as a negative control. This study indicates the power of the use of SNPs for studies not only of LOH, but also gene mapping, linkage disequilibrium and haplotype mapping.

Examples of the Plurex™ Multiplex PCR System for Genotyping Analysis Platforms: Primer Extension and Microsatellite Analysis. *A.P. Sassi, M. Satya, D. Albagli, R. Anderson.* ACLARA BioSciences, Inc., Mountain View, CA.

For high-throughput genotyping to be cost-effective, systems are needed which can streamline the genotyping process from crude sample through to genetic information. Multiplexing can accomplish higher throughput by processing many samples through one stage (purification, amplification, extension, detection) at a single time. For example, a variety of analytical techniques exist in which many samples can be detected at one time, such as electrophoresis, mass spectrometry, and array hybridization.

ACLARA's Plurex™ Multiplexing System is a plastic microfluidic device designed to address high-throughput sample preparation by multiplexing PCR amplification. The device is an array of 96 spiral channels contained in a standard SBS 96-well plate footprint. Each channel contains ten spatially isolated reaction zones that perform up to 50 individual PCR amplifications in each channel. Thus, in a single device, up to 4800 amplicons can be generated.

We have used amplicons from multiplexed reactions in the Plurex device to demonstrate compatibility with several downstream genotyping platforms. Here we present data from three genotyping demonstrations: primer extension/array hybridization, primer extension/mass spectrometry, and direct analysis by capillary electrophoresis for microsatellite-based genotyping. In each case, results are compared to pooled tube-based controls.

Active L1 retrotransposons in the human genome. *B. Brouha*¹, *R. Badge*², *J. Schustak*¹, *J. Moran*³, *H. Kazazian*¹. 1) Dept Genetics, Univ Pennsylvania, Philadelphia, PA; 2) Department of Genetics, University of Leicester, Leicester, UK; 3) Dept Genetics, Univ Mich., Ann Arbor, MI.

Though most are inactive, L1 retrotransposons comprise 17% of the human genome. There are 14 known cases of an insertion causing disease. Full-length L1s are ~6kb and move by a copy and paste mechanism. According to Boissinot et al, (2000), the non-Ta category, characterized by GAG at L1 positions 5926-8, is the oldest L1 family. The pre-Ta family, with ACG at these positions, is the next oldest and the Ta family, with ACA, is the youngest. The Ta family is subdivided, from oldest to youngest, into Ta-0, Ta-1nd, and Ta-1d subfamilies. We isolated all 89 potentially active L1s (full length with 2 intact ORFs) from the sequenced human genome. Three of the 89 elements were non-Ta, 26 were pre-Ta, 60 were Ta, and 2 did not fall into any category. Of Ta elements, 17 were Ta-0 elements, 1 was a Ta-0/Ta-1 intermediate, 3 were Ta-0 elements with the Ta-1d deletion, 16 were Ta-1nd, and 21 were Ta-1d. We tested 81 of the 89 elements for presence in 23 individuals from 5 ethnic groups and found 37 were not fixed in the population, making them useful phylogenetic markers. Putative younger elements tended to be rarer than older ones. To date, 46 of the 89 have been cloned and assayed in cell culture for retrotransposition activity relative to L1RP (a highly active element). We found activity for 4 of 13 pre-Ta elements (average activity 2.6% of L1RP); 6 of 11 Ta-0 (1.9% of L1RP); 4 of 9 Ta-1nd (3.4% of L1RP); and 9 of 12 Ta-1d (24.75% of L1RP). Putative younger elements had higher average activities and greater variation in activity. Extrapolation by category indicates that 45 of the 89 elements will be active. With a finished genome we expect about 48 active elements per haploid genome or 96 in the diploid genome. This work triples the number of known active human L1s from 10 to 34. Upon completion, we will know the number, genomic location, activity level, and polymorphism information content of all active L1s in the sequenced human genome. The data provide further evidence that L1s continue to retrotranspose, causing genomic expansion and insertional mutagenesis.

A Novel Structural Domain in the L1 Retrotransposon. *A.H. Farley, H.H. Kazazian Jr.* Department of Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA.

LINE-1 (L1) retrotransposons constitute ~17% of the human genome and are thought to integrate into genomic DNA through an endonuclease (EN) nick and target primed reverse transcription (RT) mechanism. We identified a mutation at residue 364 of L1 ORF2 in an uncharacterized region between the EN and RT domains that had an effect in reducing retrotransposition activity and insertion length in a cell culture based assay of retrotransposition. Iterative BLAST searches (PSI-BLAST and XBLAST), resulted in several significant hits to proteins rich in α -helices. This finding was reinforced by 2 structure prediction programs (COILS and PHD), which divided residues 318 to 374 into three helices, the first having a high probability of forming a coiled-coil motif. This arrangement is a general hallmark of helix-turn-helix DNA binding motifs, but especially homeobox proteins. A ClustalW alignment using this segment of L1 and human HoxB1 resulted in 35% identity. We used this alignment to generate a three-dimensional model of the L1 segment using the homology-modeling program MODELLER 6. The model was verified using the energy profile program PROSAAII and conformation validation program PROCHECK. A three-dimensional alignment of the L1 model with the template HoxB1 structure had a root mean squared deviation of less than 1.0 Å. We made six selective mutations based on the L1-HoxB1 alignment two unconserved mutations and four conserved mutations. We assayed the ability of the six mutants and the wild type (wt) parental construct (L1_{RP}) to retrotranspose in the cell culture assay using the EGFP retrotransposition marker and 143B osteosarcoma cells. The two unconserved mutants A334W and N360A have 100% of wt activity. The four conserved mutants L324P, K342A/I343A, R364G, E369A/R370A have 9, 4, 60, and 8% of wt activity respectively. The contrast between the effects of mutating conserved versus unconserved residues points towards a relationship between L1s and homeobox/helix-turn-helix proteins.

The A-tail length controls active SINE elements in populations. *P.L. Deininger¹, A.M. Roy-Engel¹, A.-H. Salem², T. Oyeniran¹, L. Deininger¹, N. West¹, D.J. Hedges², G.E. Kilroy², M.A. Batzer².* 1) Tulane Cancer Center, New Orleans, LA; 2) Dept. of Biology, LSU, Baton Rouge, LA.

There are over 1 million Alu inserts in the human genome, yet very few of these elements are capable of amplification. Most recent Alu inserts in the human genome have very long A-tails. Because the A-tails are coded from the source Alu element, this observation suggests that only Alu elements with A-tails greater than 40 bases in length may be capable of amplification. There are only a few hundred such Alu elements in any given genome. Furthermore, the long A-tails seem to be evolutionarily unstable. However, although the tendency is for the A-tails to shorten, older Alus with shorter tails may also sporadically increase their A-tail length and become reactivated. These findings help explain the amplification dynamics and subfamily structure of SINEs. We also find that Alu RNAs with these long A-stretches are in RNP complexes with poly A binding protein (PABP). We describe a model which explains both the population dynamics of these active elements and the role of the long A-tail and PABP in the amplification process.

Molecular evolution of human LINE-1 retrotransposons since before the human-chimpanzee divergence. *G.D. Swergold, A. Rubin, I. Ovchinnikov.* Columbia Univ, New York, NY. 10032.

The goal of this study was to fill a large gap in our knowledge of the evolution of L1 elements in the human genome. This gap stretched from the time of divergence of the gorilla and human lineages, usually estimated as between 8 and 11 million years ago, till the rapid expansion of the human-specific L1Hs-Ta elements approximately 2 million years ago. Our approach relied on the use of shared sequence variants (SSVs) that differentiate the currently amplifying L1Hs-Ta elements from the last insertions that amplified prior to the hominid-chimpanzee divergence (L1PA2). We performed a series of 12 BLATN searches against GenBank using a query sequence derived from the L1 3' UTR that contained various combinations of the SSVs. A total of 155 insertions that matched the query sequences were identified. These could be separated into several groups of insertions that are estimated to be between 2 and 8 million years old. The relationships among the groups was investigated by human population and species-level genotyping, and by structural studies (insertion length, poly-A tail, open reading frames), sequence alignments, and phylogenetic analyses. Our results indicate that the new L1 groups account for much of the molecular evolution of human L1s since the time of the hominoid-great ape divergence. We report the first identification of a L1 subfamily that amplified both before and after the divergence of humans from our closest relatives. Our data are consistent with the hypothesis that the rate of L1 amplification has been increasing during recent human evolution.

Characterization of Mutations in Severe Methylenetetrahydrofolate Reductase Deficiency Reveals an FAD-Responsive Mutation. *S. Sibani, E. O'Ferrall, C. Artigas, D. Rosenblatt, R. Rozen.* Human Genetics, Pediatrics and Medicine, McGill University, Montreal, Quebec, Montreal.

Methylenetetrahydrofolate reductase (MTHFR) synthesizes 5-methyltetrahydrofolate, a major methyl donor for homocysteine remethylation to methionine. Severe MTHFR deficiency results in marked hyperhomocysteinemia and homocystinuria. Patients display developmental delay and a variety of neurological and vascular symptoms. Cloning of the human cDNA and gene has enabled the identification of 24 rare mutations in homocystinuric patients, and two common variants (677CT (A222V) and 1298AC (E429A)) with mild enzymatic deficiency. Homozygosity for 677CT or combined heterozygosity for both polymorphisms is associated with mild hyperhomocysteinemia. In this communication, we describe three novel mutations in patients with homocystinuria: a missense mutation (1025TC, M338T), a nonsense mutation (1274GA, W421X) and a 2-bp deletion (1552delAG). We expressed the afore-mentioned missense mutation as well as 2 previously-reported amino acid substitutions (983AG (N324S), and 1027TG (W339G)) and observed decreased enzyme activity at 10%, 36%, and 21% of control levels, respectively, with no effect on affinity for 5-methyltetrahydrofolate. One of these mutations, 983 AG (N324S) showed FAD responsiveness in vitro. Expression of these mutations in cis with the 677CT polymorphism, as observed in the patients, resulted in an additional 50% decrease in enzyme activity. This report brings the total to 32 severe mutations identified in patients with severe MTHFR deficiency.

Novel mutations in the AASS gene and molecular consequences in patients with Familial Hyperlysinemia. *N. Braverman¹, L. Chen¹, L. Bowe¹, N.M. Kim¹, E. Christenson², A. Lossos³, M.T. Geraghty⁴*. 1) McKusick-Nathans Inst Genet Med, Johns Hopkins Univ Sch Med, Baltimore, MD; 2) Rigshospitalet, Copenhagen, Denmark; 3) Hadassah Univ Hospital, Jerusalem, Israel; 4) CHEO, Ottawa, Canada.

Familial Hyperlysinemia (FH) is a rare autosomal recessive disorder characterized by elevated lysine in blood, urine and CSF. The relationship of hyperlysinemia to a clinical phenotype is unclear, as mental retardation, neurological abnormalities and asymptomatic individuals have been described. In mammals, the major lysine catabolic pathway requires the bifunctional enzyme, α -amino adipic semialdehyde synthase (AASS), which has both α -ketoglutarate reductase (AKR) and saccharopine dehydrogenase (SDH) activities. We previously identified the AASS gene and reported a homozygous frameshift mutation in a child with mental retardation and lens dislocation.

We now report 7/8 possible AASS mutant alleles in 4 additional probands with hyperlysinemia (R132H/R132H, R910P/R910P, L602X/L602X, L804P/unknown). We show by RTPCR that the normal AASS gene is alternatively spliced in a tissue dependent pattern, resulting in either a full-length transcript, a transcript encoding SDH only, or both. We suggest that the variable FH phenotypes might result from different effects of the mutations on the encoded transcripts and thus different residual activities of AKR and SDH. In *S. cerevisiae*, AKR and SDH are synthetic enzymes with significant amino acid identity to AASS. We are evaluating an assay in yeast deletion strains *lys1* (AKR), *lys9* (SDH) and the double mutant by transforming these cells with human AASS constructs that include the patient mutations and determining their growth ability on lysine free media. Since LYS1 is a peroxisomal matrix protein, we are determining the intracellular localization of epitope tagged AASS and the putative AKR and SDH proteins in mammalian cells.

Outcome of Child Protective Services referrals in children with PKU and other inborn errors of metabolism. *G.L. Arnold, J.M. DeLuca, E.M. Blakely.* Division of Pediatric Genetics, University of Rochester School of Medicine and Golisano Children's Hospital, Rochester, NY.

Since 1990 the Inherited Metabolic Disorders (IMD) Clinic in our institution has made 14 Child Protective Services (CPS) referrals on behalf of 8 children with PKU, 2 with OTC, and 1 with biotinidase deficiency. Indications included failure to thrive and poor management (1 PKU, 2 OTC) elevated phenylalanine (phe) levels (7) and failure to administer biotin (1). Three repeat referrals were required. No children were removed from a parent's home solely for poor metabolic disease control, but as a result of the CPS home investigation 2 children with PKU were permanently removed to foster care for severe abuse/neglect and another father was jailed for sexual abuse. A fourth PKU child was temporarily removed to grandmother, and a fifth was permanently removed to father. For the PKU group, there was a significant and sustained improvement in metabolic control in the referred children. In this group mean age at referral was 6.5 years (range 8 mos-11 yrs), and mean phe level for the six months before the referral was 15.2 mg/dl. Mean phe level for the six months after the referral was 11.4 mg/dl ($p=0.05$), and from 6-12 months after referral it was 9.7 mg/dl ($p<0.04$). For the 2 children with OTC one family moved away after a second referral by our clinic, and the child was later removed to foster care after a CPS referral initiated by the new IMD clinic found physical abuse. The other child died during an episode of hyperammonemia. The child with biotinidase deficiency experienced developmental delays that improved after CPS ensured consistent biotin supplementation.

We found that poor metabolic disease control rarely existed alone, and was nearly always a function of other psychosocial problems in the home. For the children with PKU, CPS intervention resulted in sustained improvement in disease control. Although referrals to CPS are time-consuming and frustrating, we found that children with metabolic disorders benefited from them. In addition several children were rescued from severe abuse/neglect that had not been previously recognized.

Carrier and Newborn Diagnostic Test for the Common Y438N Maple Syrup Urine Disease Allele by DHPLC.

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Maple Syrup Urine Disease (MSUD) is a recessive metabolic disorder resulting from defects in the branched chain α -keto acid dehydrogenase complex (BCKAD). Infants with classic MSUD appear normal at birth, become symptomatic within 3-7 days, and may die within 2-3 weeks if untreated. Although generally rare (1:200,000 births), the incidence of MSUD in certain Mennonite communities is high (1:150 live births), and results from a specific defect (Y438N; previously referred to as Y393N) in the BCKAD E1a subunit gene. Accumulation of branched chain amino acids in infants with MSUD is necessary for diagnosis by classic serum amino acid analysis, increasing the risk of the infant to becoming clinically symptomatic. Diagnosis within the first few hours of life would allow early treatment and improved prognosis for infants homozygous for the Y438N E1a defect. We describe development of a new DNA-based PCR-DHPLC (denaturing high-performance liquid chromatography) assay for the Y438N BCKADHA allele (E1a gene), with a turn around time of 4 hours. Genomic DNA from normal control, heterozygote, and Y438N homozygous individuals were amplified by PCR, the PCR products denatured, and allowed to gradually reanneal. PCR products were then separated using a WAVE™ DNA Fragment Analysis System (Transgenomic). We could easily distinguish heterozygotic individuals from homozygotic individuals by partial denaturation and with maximum separation at 61°C. To resolve homozygous Y438N individuals from normal control individuals we modified the DHPLC protocol to include two additional analyses in which the PCR product to be tested was also mixed in a 1:1 ratio with the PCR product from (1) a normal control individual and (2) a homozygous Y438N individual, prior to denaturation and reannealing. This allowed for detection of all three genotypes, and makes this technology applicable to newborn diagnosis as well as carrier testing for the Y438N E1a defect responsible for MSUD in the Mennonite community.

The investigation of genetic background effects in mouse phenylketonuria by transgenesis: Altered blood phenylalanine levels and susceptibility to epileptic seizures. *D. McDonald, K. Panchapakesan, V. Velamakani.* Dept Biological Sci, Wichita State Univ, Wichita, KS.

We have undertaken a study of the contribution of genetic background effects to the phenylketonuria phenotype in the genetic mouse model for phenylketonuria BTBR/Pas-Pah^{enu2}. To accomplish this, we have introgressed the Pah^{enu2} mutation, originally induced in the BTBR/Pas genetic background, onto the C57Bl/6J genetic background through a classical transgenic mating strategy.

By the time that the C57Bl/6J-Pah^{enu2} transgenic line was four generations away from the BTBR/Pas background, several phenotypic aspects of the phenylketonuria syndrome were noted as being distinctly different. The hallmark phenotype of phenylketonuria is elevated blood phenylalanine levels. The average blood phenylalanine level is significantly higher ($P < 0.0001$) in C57Bl/6J-Pah^{enu2} homozygous mutants (1601 ± 57 mM) than in BTBR/Pas-Pah^{enu2} homozygotes (1139 ± 53 mM). Another phenotypic trait that is observed in untreated human phenylketonuria is epileptic seizures. This trait is not seen in untreated BTBR/Pas-Pah^{enu2} homozygotes. However, untreated C57Bl/6J-Pah^{enu2} homozygous mutants are prone to such seizures upon tactile stimulation. Of 25 homozygous mutants produced to date, 13 have been observed to experience a seizure at least once. Of those that have been observed to seizure, over half experience seizures on a daily basis. The age of the observed onset of seizuring ranges from 340 days to 559 days.

The C57Bl/6J-Pah^{enu2} transgenic line is currently eight generations away from the BTBR/Pas background. It is clear from our results that the phenylketonuria phenotype is more severe in several important respects on the C57Bl/6J genetic background than on the BTBR/Pas background. Having discovered this, our goal is to define the genetic basis of this difference. We are especially interested in seeking out the specific genes that modify the phenylketonuria phenotype.

Program Nr: 1409 from 2002 ASHG Annual Meeting

A short program for detection and quantification of isoleucine, leucine, valine, phenylalanine and tyrosine using a Beckman 6300 amino acid analyzer. *P.A. Levy.* Center for Congenital Disorders, Children's Hospital at Montefiore, Bronx, NY.

Quantification of physiologic amino acids using a Beckman 6300 amino acid analyzer is generally done using a 3 buffer (Li-A, Li-B, Li-C) or 4 buffer (Li-A, Li-D, E and a mixture of Li-C and Li-F) method. These methods usually require about 2 hours per run plus recycling time.

Management of patients with newly diagnosed Maple Syrup Urine Disease (MSUD) and Phenylketonuria (PKU) requires frequent monitoring of plasma amino acid levels. For PKU monitoring Phenylalanine and Tyrosine levels is generally sufficient. For patients with MSUD, levels of Leucine, Isoleucine and Valine should be monitored as well as measurement of Allo-isoleucine is needed initially on an frequent basis. Periodically, the full run of amino acids is necessary to correctly manage these patients.

This abstract reports a short method (26 minutes) that quantitates the levels of Valine, Isoleucine, Leucine, Phenylalanine, Tyrosine, and reliably detects Allo-Isoleucine, which co-elutes with another amino acid and is difficult to detect on the longer runs. Comparisons were done with split samples comparing the short run with the full run and showed good correlation for the amino acids measured. Norleucine was used as an internal standard to account for injection errors.

Chronological change of serum amino acid pattern and mechanism of citrullinemia in infancy in a patient with citrin deficiency. *M. Yoshino¹, I. Yohida¹, K. Kobayashi², T. Shinka³, T. Kuhara³, T. Saheki².* 1) Dept Pediatrics & Child Health, Kurume Univ Sch Medicine, Kurume, Japan; 2) Depart of Biochem, Faculty of Medicine, Kagoshima Univ, Kagoshima, Japan; 3) Division of Hum Genet, Medical Research Institute, Kanazawa Medical Univ, Kanazawa, Japan.

Deficiency of citrin, the mitochondrial aspartate/glutamate carrier, causes adult-onset type II citrullinemia in adulthood, whereas in infancy it presents with cholestatic liver disease as transient citrullinemia. Chronological change in serum amino acid pattern in a female patient with this disease was studied from the neonatal period up to twenty years of age. Findings suggested that citrin deficiency caused insufficient aspartate supply from mitochondria for argininosuccinate synthesis, and predisposed the patient to develop citrullinemia when, in early infancy, she was exposed to relatively excessive protein load. After two years of age, serum aspartate concentration continued to decrease in an age-related manner to reach a subnormal level at twenty years of age, which is likely to cause hyperammonemic crisis with citrulline elevation in conjunction with hepatic argininosuccinate synthetase deficiency. As the patient grew older, concentration of glutamate in serum decreased, whereas the ratio of concentration of glutamine to that of glutamate (Gln/Glu ratio) increased. This observation implies that diversion of glutamate to glutamine synthesis increased as the patient grew older and it limited supply of glutamate available for aspartate export out of mitochondria. Such a decrease in glutamate supply in mitochondria could still be compatible with normal aspartate export required for normal argininosuccinate synthesis in healthy individuals, but such a decrease in aspartate export could be rate-limiting in argininosuccinate synthesis in patients with citrin deficiency. Conclusion: In citrin deficiency, insufficient aspartate supply predisposes the patient to develop citrullinemia in the early infancy when the protein load is superimposed, and can be a precipitating factor for hyperammonemic crisis with citrullinemia in adulthood.

Treatment experience of neonatal hyperammonemia. *W. Shu-Mei, J. Hou, J. Lin.* Division of Medical Genetics, Chang Gung Children's Hospital, Taoyuan, Taiwan.

Hyperammonemia associated with inherited disorders of amino acid and organic metabolism is usually manifested by irritability, somnolence, vomiting, seizures, and coma. The majority of these patients present in the newborn period, and the aim of therapy should be to normalize blood ammonia levels. Treatment guidelines include minimizing endogenous ammonia production and protein catabolism, restricting nitrogen intake, administering substrates of the urea cycle, administering compounds that facilitate the removal of ammonia through alternative pathways, and, in severe cases, dialysis therapy. Three cases of neonatal hyperammonemia were treated in our hospital during the past four years. Ornithine transcarbamylase (OTC) deficiency was suspected in the first two cases, and the last one was methylmalonic acidemia. They all had the symptoms of poor activity, poor feeding and respiratory distress. They were admitted within four days after birth and were intubated. Severe hyperammonemia and acidosis were found soon after admission. Peritoneal dialysis, continuous arteriovenous hemofiltration (CAVH), and continuous arteriovenous hemodialysis (CAVHD) had been applied to all three cases. Ammonia decreased after treatments. But eventually they died. Before they died, multiple bullae and generalized erythema were found. The causes of death and the dermatological changes will be discussed. Besides, the effects of different therapies during the acute phase of hyperammonemia will be analyzed.

Program Nr: 1412 from 2002 ASHG Annual Meeting

Effects of liver transplant & bone marrow transplant upon amino acid homeostasis & the neuropathology of maple syrup disease. *D.L. Robinson, K.A. Strauss, E.G. Puffenberger, D.H. Morton.* Clinic for Special Children, Strasburg, PA.

The Clinic for Special Children was founded in 1988 as a non-profit medical clinic for Amish & Mennonite children who have genetic disorders. We now follow more than 80 patients with classical maple syrup disease (MSD). In patients with MSD acute imbalances in plasma amino acid levels provoke vomiting, encephalopathy, movement disorders, behavioral changes, focal and generalized brain edema while chronic imbalances, especially during infancy, cause poor brain growth & mental retardation. Liver is the primary site of regulation of the plasma neutral amino acid concentration ratios and these ratios determine the rate of uptake of essential amino acids into brain. A 9 year old patient of ours with MSD underwent liver transplant for hepatic failure. At the time of transplant her neurological findings included stupor, irritability, ataxia, tremor, intermittent dystonia, hyperreflexia with + Babinski signs. Her plasma amino acid profiles were markedly abnormal because of advanced liver failure and GI hemorrhages. Five years after liver transplant her neurological examination is normal. On an unrestricted protein intake & during catabolic illnesses, her plasma amino profiles & the calculated brain uptake rates for neutral amino remain normal. We have recently managed a neonate with MSD & severe combined immune deficiency. At one week of age he was lymphopenic (ALC <100/mm) & markers were negative for T-lymphocytes & positive for B-cell & natural killer cells. An uncomplicated bone marrow transplant was done with marrow from from an HLA matched normal sibling. Within 6-12 months after bone marrow transplant we predict a significant number of hepatocytes with normal BCKD activity will have developed from donor stem cells transplanted with the bone marrow. Detailed records of his tolerance of dietary leucine, plasma neutral amino acid ratios, and molecular markers have provided us with a unique opportunity to study the effects of bone marrow transplant upon amino acid homeostasis in MSD. These observations may have important implications for the use of bone marrow or hepatic stem cell transplants to treat such disorders as Crigler-Najjar disease and PKU.

PHENOTYPIC VARIABILITY OF CARNITINE PALMITYL TRANSFERASE I DEFICIENCY (CPT I)

WITH NOVEL MOLECULAR DEFECT IN SAUDI ARABIA. *A.I. Al-Aqeel¹, M.S. Rashed², L. Ijst³, J.P.N.*

Ruiter³, R.J.A. Wanders³. 1) Dept of Pediatrics, Riyadh Military Hospital, Riyadh, Saudi Arabia; 2) Dept of Genetics, King Faisal Specialist Hospital & Research centre, Riyadh ,Saudi Arabia; 3) Dep of Clinical Chemistry, University of Amesterdam, The Netherlands.

Carnitine palmityl transferase I is the key enzyme in the carnitine dependent transport of long chain fatty acids across the mitochondrial inner membrane and its deficiency results in a decrease rate of fatty acids b-oxidation with decreased energy production. We report two families of five affected patients who are the product of first degree cousin marriage. Two patients presented with typical Reye-like syndrome with unconsciousness, hepatomegaly, hypoglycemia, hyperammonemia and very high liver enzymes with hemophagocytic syndrome and brain abscess in the second patient. Liver biopsy showed steatosis. On screening of the two families, the other three siblings were found to have hepatomegaly. The five siblings showed an acyl carnitine profile with very high free carnitine with almost absent long-chain acyl carnitines , suggestive of CPT-I deficiency. This was confirmed by enzyme analyses in fibroblast cultures. Molecular analysis on cDNA shows 1950 GoA transition resulting in glycine 650 aspartic acid change (G650D) in the protein. These patients were effectively treated with a diet high in carbohydrate, low in long chain fatty acids with medium chain triglycerides. In conclusion, CPT -I deficiency is an important cause of Reye-like syndrome with secondary hemophagocytic syndrome, which may be treated easily with very good results if detected early in life.

Molecular and biochemical diagnosis of lysosomal free sialic acid storage disorders. *D.L. Fitzpatrick¹, R. Kleta¹, R.A. Martin², D.J. Aughton³, M.J. Rivkin⁴, E. Orvisky⁵, M. Huizing¹, D. Krasnewich⁶, W.A. Gahl¹.* 1) Section on Human Biochemical Genetics, HDB, NICHD, NIH, Bethesda, MD; 2) Children's Hospital, Washington University, Saint Louis, MO; 3) William Beaumont Hospital, Royal Oak, MI; 4) Children's Hospital, Harvard Medical School, Boston, MA; 5) NSB, NIMH, NIH; 6) MGB, NHGRI, NIH.

The differential diagnosis of developmental delay in childhood is difficult and challenging. The allelic lysosomal storage disorders Salla disease and infantile free sialic acid storage disease (ISSD) can cause such developmental delay. These patients have mutations in the SLC17A5 gene which codes for the lysosomal transporter sialin, responsible for the egress of the charged sugar, sialic acid (SA), out of lysosomes. We describe 3 North American (non-Finnish) sibships with different degrees of lysosomal free SA storage disease. 5-year old twins had developmental delay, hypotonia, mildly coarse facial features, truncal ataxia, and poor speech and ambulation. The free SA content of fibroblasts was 7.7 ± 3.1 nmol per mg protein (normal 1.1 ± 1.0), and cDNA mutation analysis revealed homozygosity for the typical Salla mutation, R39C. A female infant, normal at 4 weeks of age, had progressive growth retardation, developmental delay, seizures, coarse facies, hepatosplenomegaly, cardiomyopathy and fatal respiratory insufficiency at 33 months of age. Fibroblasts contained 86 ± 30 nmol free SA per mg protein, and the patient was compound heterozygous for a deletion of exon 9 and a 15-bp deletion in exon 6 of sialin. A fourth patient had developmental delay intermediate in severity between that of patients 1,2 and 3. Fibroblasts contained 9.1 ± 1.1 nmol free SA per mg protein, and the patient was compound heterozygous for the Salla mutation and the above mentioned 15-bp deletion. These 3 sibships illustrate that the clinical manifestations of different types of lysosomal free SA storage diseases correlate with biochemical findings (fibroblast free SA content) and with sialin mutations. Thus, the differential diagnosis of postnatal developmental delay should include free SA storage disorders such as ISSD and Salla disease regardless of ethnic background.

The Identification of Different Tissue Isoforms of Glucocerebrosidase in Patients With Gaucher Disease. E.

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Gaucher disease (GD) is an autosomal recessive storage disorder caused by mutations in the gene encoding for the lysosomal enzyme glucocerebrosidase (GC). There are three clinical variants of the disease: type 1, chronic non-neuronopathic; type 2, acute neuronopathic; and type 3, subacute neuronopathic. Genotype-phenotype correlations among the three types are limited. To discriminate between the neuronopathic and non-neuronopathic phenotypes of GD, multiple isoforms of GC have been studied in fibroblasts. Previous studies of glucocerebrosidase have shown that there are four glycosylation sites on the protein. Western blots of fibroblast GC have consistently shown three isoforms with molecular weights of 63, 61, and 59kDa, where the 59kDa form is believed to be the functional GC. We have done a more extensive study on GC in multiple tissues from patients with Gaucher disease by comparing brain, liver, and spleen isoforms of GC in the same patients. Brain GC showed different isoforms than the other tissues analyzed. Westerns performed on samples of brains from normals consistently demonstrated two bands for GC, one at 56kDa and one at 59kDa. Brain samples from patients with Gaucher disease predominantly had the 56kDa form, but were all deficient in the functional 59kDa form. Thus, it is possible that neurologic involvement in GD may correlate with the lack of the functional form of the enzyme in the brain. Tissue extracts were then digested with Peptide-N-Glycosidase F (PNGase) to remove all oligosaccharides. After PNGase digestion, all samples demonstrated only the lowest 56kDa form of glucocerebrosidase regardless of the tissue studied. This form must be minimally glycosylated or unglycosylated and therefore the alternate forms of GC observed in brain are most likely due to alternative or absent glycosylation.

Towards the diagnosis of defects in the biosynthesis of glycosaminoglycans. *N. Leeuw, de¹, A. van Kuppevelt², A. Oosterhof², S. Wopereis³, C.M. Huyben³, R.A. Wevers³.* 1) Human Genetics, University Medical Centre Nijmegen, Nijmegen, The Netherlands; 2) Department of Biochemistry, University of Nijmegen; 3) Institute of Neurology, University Medical Centre Nijmegen.

Glycans (present in glycoproteins and proteoglycans) are complex molecules fulfilling a wide range of activities essential for a variety of biological processes such as morphogenesis, transport and signalling of biomolecules and immune response. Several glycan degradation defects are known and (sub)grouped together in the so-called Storage Diseases. So far, very little is known about defects in the biosynthesis of glycan molecules.

Defective glycan molecules are suspected in a specific category of patients who primarily suffer from bone and skin malformations. Identification of such defective glycans in these patients will facilitate the search of the underlying genetic defect.

In order to be able to investigate whether certain glycosaminoglycans are absent or malformed in patients with bone and / or skin anomalies, we developed an assay with immunofluorescently labelled antibodies that specifically recognise certain carbohydrate moieties of heparan sulphate (HS), one of the glycosaminoglycans.

Preliminary data of the Immuno Fluorescence Assay have shown that five anti-HS antibodies seem to specifically bind to blood cells that are known to express HS on their cell surface. The applicability of this assay is verified by analysing samples and cell lines from several patients who suffer from bone dysplasia due to an identified gene defect. Subsequently, we will analyse blood and urine samples from patients with progeria and / or bone dysplasia (gene defect unknown).

The use of antibodies that specifically recognise the carbohydrate moieties of certain proteoglycans seem to provide an appropriate screening approach to identify aberrant glycan molecules in patients with bone and skin malformations.

Further studies on the Arginase I-deficient mouse. *R.K. Iyer*^{1,5}, *H. Yu*^{3,5}, *R.M. Kern*^{3,5}, *P. Yoo*³, *R.W. Tsoa*³, *W.W. Grody*^{1,2,4,5}, *W.E O'Brien*⁶, *S.D Cederbaum*^{2,3,4,5}. 1) Pathology & Lab Med; 2) Pediatrics; 3) Psychiatry; 4) Human Genetics; 5) Mental Retardation Research Center, David Geffen School of Medicine at UCLA, Los Angeles, CA; 6) Molecular and Human Genetics, Baylor College of Medicine, Houston TX.

Deficiency of hepatic arginase (AI) is characterized (in humans) by progressive mental impairment, growth retardation, spasticity, and punctuated episodes of hyperammonemia. The AI-deficient mouse (Iyer et al, 2002, *Mol Cell Biol*, 22:4491-98) replicates several pathobiological aspects of the human condition, but (unlike humans) dies at day p10-p12 exhibiting severe hyperammonemia and liver failure. Here we report the results of some further comparative studies on pre-symptomatic (p6-p9/10) and symptomatic (p10-12) AI-deficient mice, versus their normal (wildtype or heterozygote) counterparts: (a) GROWTH RATE:- AI-deficient mice were ~15% smaller at birth; in addition, significant and increasing growth retardation was observed in the pre-symptomatic period; (b) PLASMA AMMONIA:- Moderate hyperammonemia was apparent in AI-deficient mice by day p6-p8 and reached symptomatogenic levels by p10-12; (c) PLASMA AMINO ACIDS:- Hyperargininemia, hypoornithinemia and hypoprolinemia, all predictable consequences of lack of AI activity were seen in p6-7 day old AI-deficient mice. Though plasma arginine remained steady at a high level and proline dropped further, a seemingly paradoxical increase in ornithine concentration was observed in symptomatic versus non-symptomatic AI-deficient mice, suggesting the possibility that (unlike other urea cycle disorders) ornithine deficiency may not play a major role in the pathology of this disorder. Glutamate was also increased in symptomatic mice, compared to non-symptomatic AI-deficient and normal mice. SUMMARY:- Like in hyperargininemic patients, growth retardation and hyperammonemia were seen in the AI-deficient mice even before plasma ammonia reached lethal levels. In addition, the increased glutamate levels seen in symptomatic animals could mediate excitotoxic neuronal injury, a pathologic process that may also be implicated in the progressive neurological impairment seen in human patients.

Galactose-1-phosphate uridylyltransferase deficiency causes decreased UDP-hexose concentrations in human galactosemic cells. *K. Lai¹, S.D. Langley¹, F. Khwaja², E.W. Schmitt¹, L.J. Elsas^{1,2}.* 1) Dept Ped, Div Medical Genetics, Emory Univ, Atlanta, GA; 2) The GMB program. Grad Sch of Arts & Sci, Emory Univ, Atlanta, GA.

Previously we reported that stable transfection of human UDP-glucose pyrophosphorylase (hUGP2) rescued galactose-1-phosphate uridylyltransferase (GALT) deficient yeast. Here we test the hypothesis that excess accumulation of galactose-1-phosphate (gal-1-P) in fibroblasts from galactosemic patients inhibits hUGP2 and creates UDP-hexose deficiency. We transfected SV40 immortalized fibroblasts from a galactosemic patient homozygous for the Q188R mutation (GALT deficient) and a control with pCDNA3.0 containing either hUGP2 or hGALT. Stable neomycin-resistant transfectants had hUGP2 or hGALT activity at least 2 fold greater than the background. We quantitated critical metabolites in this evolutionarily conserved pathway in all four cell lines before and after two days in galactose media (5.5mM). Galactosemic cell lines accumulated gal-1-P from 1.2 ± 0.4 to 5.7 ± 0.5 mM ($p < 0.01$) when cultured in D-galactose and ceased to grow. Control and transfected cell lines had about 30% reduction in UDP-glucose (UDP-glc) and UDP-galactose (UDP-gal) concentrations when transferred to D-galactose. Untransfected GALT-deficient cells had the lowest UDP-hexoses and highest gal-1-P in both media. When transfected with hUGP2 or hGALT, GALT-deficient cell lines had a 40-100% increase in UDP-glc and UDP-gal even in D-galactose. Using purified hUGP2 we found that the K_M for glucose-1-phosphate increased from 19.7mM to 1900mM in the presence of 2.5mM gal-1-P. We conclude that gal-1-P is toxic to human cells and that its pathologic mechanism results in reduced cellular content of UDP-hexoses that are essential for human fibroblasts growth and survival.

Variant non-lethal rhizomelic chondrodysplasia punctata with normal plasmalogen and cholesterol biosynthesis.

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The most common form of rhizomelic chondrodysplasia punctata (RCDP) is an autosomal recessively inherited disorder that is diagnosed in the newborn period based on classic clinical, radiologic, and biochemical features. Clinically, affected newborns typically demonstrate severe proximal shortening of all limbs, bilateral cataracts, and a characteristic facial appearance, and radiologically, epiphyseal calcifications, as well as growth failure and mental deficiencies. Biochemically, their red blood cells demonstrate a marked deficiency of plasmalogens, increased plasma concentration of phytanic acid, deficiency of the enzyme dihydroxyacetone phosphate acyltransferase and dihydroxyacetone phosphate acyltransynthase, and normal concentrations of very long chain fatty acids. In the past, variant forms of RCDP have been described in which plasmalogen synthesis has been normal. We report an individual who at birth resembled clinically classic RCDP: features included rhizomelic shortening of the arms and legs, and a skeletal survey demonstrated multiple anomalies with punctate mineralization in the epiphyses of the knees, limbs, hips, and spine. He also had a short neck with nuchal fullness, a barrel shaped chest, and bilateral brachydactyly of the hands with short 2nd and 3rd fingers. Plasmalogen synthesis, LCFA's and phytanic acid were normal. Cholesterol biosynthesis appeared normal, ruling out a milder, X-linked dominant form of chondrodysplasia (CDPX2). Over the course of a ten-month follow-up, the rhizomelia has decreased, but the disproportionate growth of the patient's digits remains striking. He has slight motor delays, but otherwise his development is appropriate for age in contrast to patients with classic RCDP. We reviewed the literature of primary and secondary CDP. Our patient does not resemble previously described cases and may represent another variant of non-lethal RCDP.

Cystinosis in Denmark: Mutation analysis and treatment with cystamine, the disulfide of cysteamine. *F. Skovby¹, R. Kleta², Y. Anikster², R. Christensen¹, W.A. Gahl².* 1) Department of Clinical Genetics, Rigshospitalet, Copenhagen, Denmark; 2) Section on Human Biochemical Genetics, HDB, NICHD, NIH, Bethesda, MD.

Nephropathic cystinosis is an autosomal recessive defect of intracellular cystine transport across the lysosomal membrane. Most patients present as infants with failure to thrive and renal tubular Fanconi syndrome. Untreated, they succumb to renal failure at ~10 years of age. Early, diligent cystine-depleting therapy with a form of cysteamine prevents or delays renal failure and other serious organ dysfunction. Cystinosis results from mutations in the gene CTNS; the most common CTNS mutation is a 57-kb founder deletion thought to arise in Germany ~500 A. D.. We reviewed the clinical course, management, and molecular genetics of 9 affected Danish children diagnosed 1969-2000. Assuming complete ascertainment, the incidence was 1:200,000 live births. Thirteen of 18 CTNS alleles contained the 57-kb deletion. Two of the 9 children, sibs diagnosed with nephropathic cystinosis after age 5, are compound heterozygous for the 57-kb deletion and a novel Alu Y repeat insertion which changes a splice site at the intron 6-exon 7 boundary. Another patient was homozygous for a novel 1007 del G nonsense mutation. Three children with nephropathic cystinosis children never received cystine-depleting therapy and died before age 11 of renal failure. All but one of the remaining patients were treated with oral cystamine, the disulfide of cysteamine, between 8 and 28 months of age and continuing for 8-16 years. All are alive. One received a renal transplant at age 13. The other 3 have glomerular filtration rates of 22, 42, and 98 ml/min/1.73m² at 19, 11, and 10 years of age. Of the 2 sibs with the Alu Y insertion on one allele, one received no cystamine and lost all renal function at age 12. The other received cystamine for 10 years starting at age 8 and went to renal failure at age 18. We conclude that the 57-kb deletion in CTNS is common in Denmark, and that cystamine, which can be prepared as a tasteless liquid for oral use, is an effective alternative to other cystine-depleting agents for treating cystinosis patients.

Functional and structural characterization of disease-causing mutations in α -mannosidosis. *H.M.F. Riise Stensland^{1,2}, G.M. Hansen¹, P. Heikinheimo^{1,3}, O. Nilssen², O.K. Tollersrud¹.* 1) Dept. Medical Biochemistry, Univ.of Tromsø, Tromsø, Norway; 2) Dept. Medical Genetics, Univ. Hospital of Northern Norway, Tromsø, Norway; 3) Dept. Chemistry, Univ.of Tromsø, Tromsø, Norway.

α -Mannosidosis is an autosomal recessive storage disorder caused by deficiency of lysosomal α -mannosidase (MAN2B1, LAMAN). Major symptoms are progressive mental retardation, skeletal changes and immunodeficiency. Previously we reported 26 putative α -mannosidosis causing mutations, of which about two thirds were severe-type mutations, while the remaining were predicted to cause single-amino acid substitutions or small in-frame deletions. To better understand the biochemical consequences of the latter group of mutations, eleven missense mutations and two small in-frame deletions were constructed by site-directed mutagenesis of human LAMAN cDNA, and expressed in transfected COS-cells. Pulse-chase analysis and intracellular localization studies showed that the mutations could be divided into three groups: Group A, containing seven mutations resulting in transport-arrest and accumulation in the endoplasmic reticulum; group B, one mutation leading to lysosomal targeting but abolition of α -mannosidase activity; and group C, five mutations leading to correct targeting and preservation of low, but significant, residual enzymatic activity towards the artificial substrate p-nitrophenyl α -D-mannopyranoside. We are currently expressing LAMAN-variants carrying the group C mutations in CHO-cells to investigate their activities towards natural substrates. To study the biochemical consequences of mutations in the active site, two active site mutants were constructed. Both these mutations fell into group B, showing that active site substitutions are compatible with correct sorting and stability. The recently solved 3-D structure of LAMAN was used to predict the structural consequences of the α -mannosidosis mutations. All five amino acid substitutions in group A are localized outside the active site regions, and are probably important for correct folding of the enzyme. The substitutions in groups B and C involve residues within or close to the active site, and do not seem to disrupt the folding process.

Lysosomal cystine loading increases apoptosis in normal and cystinotic fibroblasts and renal proximal tubule epithelial cells. *M.A. Park, A. Helip-Wooley, J.G. Thoene.* Human Genetics, Tulane Univ, New Orleans, LA.

Nephropathic cystinosis is a lethal disorder of lysosomal cystine storage due to defective lysosomal cystine transport, and culminates in end-stage renal disease by 10 years of age. The mechanism whereby lysosomal cystine causes this multi-systemic disorder is not known. We here report that increased lysosomal cystine causes increased apoptotic rates in both normal and nephropathic cystinotic fibroblasts, and in cultured human renal tubular epithelial cells (RPTE cells), when treated with TNF-alpha and ultraviolet B light, and analyzed by TUNEL, CaspACE, or Annexin V-FITC. Normal fibroblasts cystine-loaded with 0.5mM cystine dimethylester (CDME), and exposed to apoptotic stimuli, show an increase in the average rate of apoptosis from 9.7% \pm 3.2 to 16.8% \pm 3.5 ($p < 0.05$), with a lysosomal cystine increase from < 0.01 to 1.21 nmols cystine per mg protein. Cystinotic fibroblasts cystine-depleted with cysteamine, show a decrease in average apoptosis from 17.0% \pm 4.4 to 4.9% \pm 1.3, ($p < 0.05$), with a decrease in lysosomal cystine from 4.1 to 0.12 nmols cystine per mg protein. Fibroblasts from two cystinotic variants, benign ocular and intermediate cystinosis show apoptosis rates not significantly increased from normal fibroblasts, and averaging only 6.5% and 9.2% respectively. RPTE cells tolerate exposure to 0.25 mM CDME alone for only 6 h and show an apoptotic rate of 43.8%, compared to 5.4% in normal fibroblasts treated with 0.5 mM CDME for 17 h. RPTE cells are also more sensitive to other apoptotic triggers with or without CDME, with a 40.7% average apoptosis rate upon exposure to UV and TNF-alpha (without CDME), compared to an average of 6.7% in normal fibroblasts. We speculate that enhanced apoptosis resulting from lysosomal cystine storage leads to inappropriate cell death, decreased cell numbers in many tissues, and hence, the nephropathic cystinotic phenotype. The variant forms may represent co-segregation of rare allele(s) that confer resistance to apoptosis, moderating the cell loss and causing the milder phenotype.

Use of cDNA Microarrays to Evaluate Molecular Mechanisms Involved in Gray Platelet Syndrome (GPS). *T. Hyman*¹, *M. Huizing*², *Y. Anikster*², *T.C. Falic-Zaccari*³, *W.A. Gahl*². 1) LCCTP, NCI, Bethesda, MD, USA; 2) Section on Human Biochemical Genetics, NICHD, NIH, Bethesda, MD, USA; 3) Division of Medical Genetics, Hospital of Western Galilee-Naharia, Rappaport Faculty of Medicine, Technion, Haifa, Israel.

The gray platelet syndrome (GPS) is a rare congenital bleeding disorder in which thrombocytopenia is associated with increased platelet size and decreased alpha-granule content. We described two siblings with GPS who had characteristic pale blue platelets lacking alpha-granules on electron microscopy. On bone marrow biopsy, one sibling, an 8-year old girl, exhibited mild myelofibrosis and extensive emperipoiesis. We used cDNA microarray technology to investigate changes in gene expression which might reveal the mechanism involved in causing this disease. The expression of approximately 6,500 genes was evaluated in fibroblasts cultured from one of the GPS patients; normal fibroblasts provided the reference standard. Genes that were differentially regulated in the GPS cells were categorized into gene clusters according to their biological function. This analysis revealed significant upregulation of selected biological processes involving the production of cytoskeleton proteins (e.g., fibronectin, thrombospondin 1 and 2, and collagen VI a). These genes, as well as certain genes involved in cell cycle progression, appear to play a role in the pathogenesis of GPS. Indeed, northern blot analyses confirmed that fibronectin, thrombospondin and MMP2 were overexpressed in GPS fibroblasts compared to the control. Moreover, immunocytofluorescence studies revealed robust fibronectin staining in GPS fibroblasts compared to normals. Our findings support the feasibility of using cDNA microarray techniques to detect distinctive and informative differences in gene-expression patterns relevant to GPS. Specifically, our studies suggest that the molecular basis for myelofibrosis in GPS involves upregulation of cytoskeleton proteins.

Hepatic veno-occlusive disease (HVOD) after high-dose chemotherapy and blood cell transplant (BCT): Association with the major hemochromatosis (HFE) mutation C282Y and modification by polymorphisms of the hepatic urea cycle enzyme, carbamyl phosphate synthetase 1 (CPS-1). A. Kallianpur¹, L. Hall³, M. Yadav³, B. Christman¹, R. Dittus¹, J. Haines², M. Summar^{2,3}. 1) Medicine, Vanderbilt Univ. Med. Ctr., Nashville, TN; 2) Mol. Phys. & Biophysics; 3) Pediatrics.

HVOD is a dose-limiting and often fatal toxicity in patients undergoing high-dose chemotherapy and BCT for malignancy. HVOD is a syndrome of increased bilirubin and either liver enlargement or weight gain >5% over baseline, occurring by Day 21 after BCT. The C282Y mutation in the HFE gene is responsible for >85% of cases of hereditary hemochromatosis. C282Y heterozygotes have elevated mean body and liver iron content (LIC). LIC has been associated with post-BCT liver dysfunction. We determined whether carriage of at least one C282Y allele conferred an increased risk of HVOD and early death (< Day 61 post-BCT) in adult BCT patients. A secondary aim was to test for interactions between the C282Y allele and the T1405N SNP of the hepatic urea cycle (HUC) enzyme CPS-1, since HUC-generated nitric oxide (NO) may regulate iron metabolism. Genotyping was successful in 161 of 200 patients after DNA extraction from stored blood and urine, PCR amplification, and SSCP or HPLC (Genomic WAVE) SNP analysis, and scoring by outcome-blinded readers. Relative risk (RR) of HVOD was significantly increased in patients carrying ³ 1 C282Y allele, adjusting for age, sex, chemotherapy regimen, CPS-1 genotype, donor type, and 3 other known HVOD risk factors (RR=3.9 with 95% CI 1.3-12.5). An allelic dose-response effect was seen with RR of HVOD in C282Y heterozygotes of 2.2 (95% CI 0.6-7.8) and RR in homozygotes of 5.8 (95% CI 1.1-29.6). Risk of early death was increased but was not statistically significant (RR=1.69; 95% CI 0.4-8.1). A significant interaction between C282Y and the T1405 form of CPS-1 was noted (p=0.04), consistent with our previous findings that HVOD risk is related to this less efficient enzyme and inversely related to HUC efficiency/NO production. These findings suggest that the C282Y allele is a risk factor for HVOD and that its effect on the liver may be augmented by CPS-1 alleles that lead to diminished HUC function.

Clinical, radiographic, biochemical and genetic delineation of pantothenate kinase associated neurodegeneration.

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The diagnosis of Hallervorden-Spatz syndrome (HSS) is based on dystonia, parkinsonism, pigmentary retinopathy and radiographic evidence of brain iron accumulation. Recently, we discovered that many patients with HSS have mutations in the gene encoding pantothenate kinase 2 (*PANK2*) and designated such patients as having pantothenate kinase associated neurodegeneration (PKAN). We compared specific clinical and radiographic features in patients with PKAN to those in HSS patients lacking defects in *PANK2*. Ninety-eight families with the diagnosis of HSS were stratified by sequencing genomic DNA for *PANK2* mutations and by clinical assessment into early onset, rapidly progressive or later onset, slowly progressive disease. Data were compared for 123 patients from 66 mutation-positive and 32 mutation-negative families. All early onset HSS patients and one-third of later onset patients were found to have *PANK2* mutations. Whereas almost all mutations in later onset patients lead to amino acid changes, early onset disease is characterized by more severe mutations. Later onset patients with *PANK2* mutations more often exhibit prominent speech and psychiatric manifestations compared to either classical patients or the mutation-negative patients. Remarkably, in all PKAN patients, a very specific brain MRI pattern of globus pallidus changes called eye of the tiger was observed. Moreover, these changes were not seen in any mutation-negative patients. Lipoprotein abnormalities have now been demonstrated in patients with PKAN, suggesting aberrant lipid metabolism, which would be consistent with the prediction of a deficiency of coenzyme A due to defective pantothenate kinase.

Arginase I in the Nervous System during Development. *H. Yu^{1,2}, P.K. Yoo^{1,3}, R. Kern^{1,2}, R. Tsoa^{1,3}, W.W. Grody^{1,3,4,5}, S.D. Cederbaum^{1,2,4,5}, R.K. Iyer^{1,3}.* 1) Mental Retardation Res Center; 2) Dept of Psychiatry; 3) Dept of Pathology; 4) Dept of Human Genetics; 5) Dept of Pediatrics; David Geffen School of Medicine at UCLA, Los Angeles, CA.

Arginase I (liver arginase, AI), the fifth enzyme of the urea cycle, catalyzing the hydrolysis of arginine to ornithine and urea, functions primarily to detoxify ammonia as part of the urea cycle. Recently, AI was found in the brain and embryo, and thus may play an important role for synthesizing ornithine, which is a precursor in the synthesis of glutamate and polyamines. In humans, inherited deficiency of AI is characterized clinically by hyperargininemia, growth retardation and progressive neurologic impairment. The purpose of this study is to elucidate the pattern of expression of arginase, ornithine decarboxylase (ODC) and ornithine aminotransferase (OAT) in the nervous system, in order to understand the role of AI in nervous system development with the expectation that this knowledge will help patients suffering from AI deficiency. Arginase, ODC and OAT expression during mouse development was detected by DIG-labeled RNA in situ hybridization. We found that AI was expressed strongly in spinal cord in the early stages of embryonic development. In the peripheral nervous system, AI co-localized with ODC in the dorsal root ganglia of the spinal nerves during embryonic stages whereas neither AI nor ODC were expressed in these tissues during postnatal development. AI was also found in sympathetic ganglia in all stages of development, and co-localized with OAT. This study suggests that AI is important in the developmental nervous system, and may regulate the ornithine supply to influence polyamines which are important in cell growth, and to influence glutamate - a neurotransmitter. The neurologic impairment due to AI deficiency may be explained by the lack of AI activity in the nervous system during development.

Keratopathy of multiple myeloma masquerading as the corneal crystals of ocular cystinosis. *I. Bernardini¹, C.R. Hermos¹, S. Blair², M.I. Kaiser-Kupfer³, R. Kleta¹, W.A. Gahl¹.* 1) Section on Human Biochemical Genetics, HDB, NICHD, NIH, Bethesda, MD; 2) Hematology / Oncology, Riverside Methodist Hospital, Columbus, OH; 3) Ophthalmic Genetics Branch, NEI, NIH.

The isolated finding of corneal crystals observed on slit lamp examination by an experienced ophthalmologist, can make the diagnosis of ocular cystinosis. This variant of cystinosis never manifests with renal or any other systemic involvement. Here we describe a 49 year old woman diagnosed with ocular cystinosis during a routine eye examination 12 years previously. A conjunctival biopsy was reported to support the diagnosis at that time, but leucocyte cystine measurements were not performed. The patient reported photophobia and increased glare for the past 5 years, and mentioned that her serum IgG had been elevated to twice the normal level for the past 12 years. She came to the NIH to participate in an ongoing study of cysteamine eye drops, a medication known to dissolve corneal cystine crystals. When the patient's eyes were examined at the NIH, the crystals looked different from those of ocular cystinosis and appeared in the epithelial and most superficial stromal layers rather than being present throughout the corneal epithelium and the entire stroma. Triplicate leucocyte cystine measurements revealed a completely normal level of 0.04 nmol half cystine per mg of protein (normal, less than 0.2). Blood chemistries revealed an elevated sedimentation rate, hemoglobin was 12.3 g/dL. The serum protein was elevated at 84 g/l; protein electrophoresis showed an elevated gamma-globulin peak, and the IgG level was twice normal at 2820 mg/dl. Blood viscosity was slightly elevated. Urine protein electrophoresis showed also elevated gamma globulins. Bone marrow biopsy confirmed the diagnosis of multiple myeloma. This case illustrates that multiple myeloma can mimic the corneal findings of cystinosis. It also emphasizes the importance of measuring the leucocyte cystine in all patients suspected of having any form of cystinosis to confirm (or to rule out) the diagnosis. The morphology and location of the crystals in the cornea can help distinguish between cystinosis and multiple myeloma.

Natural history of cardiac involvement in hemizygotes Fabry patients. *G. Altarescu¹, D. Moore², R. Schiffmann².* 1) Shaare Zedek Medical Center, Jerusalem Israel; 2) National Institutes of Health Bethesda, MD.

Objective: To determine the natural history of cardiac involvement in Fabry disease and relation of cardiac involvement to constitutive enzyme activity. **Introduction:** Fabry disease, an X-linked lysosomal storage disorder caused by deficiency of alpha-galactosidase A, is characterized by progressive intracellular lysosomal accumulation of glycosphingolipids through body including cardiovascular system. Cardiac manifestations consist of left ventricular hypertrophy (LVH), valvular involvement and cardiac conduction defects. **Material and Methods:** 86 hemizygotes, mean age 32 years were examined by a retrospective historical analysis. Mean alpha-galactosidase A levels was 0.45% of controls. Patients were genotyped. Cardiac function was evaluated by ECG and 2-D Echocardiography (ECHO) every 6 months over 3-17 years. PR, QT intervals (ms) and the septal thickness (mm), left ventricular (LV) free wall thickness (mm) and aortic root (mm) were found to be outside the normal range. Subsequent statistical analysis examined these parameters as indices of cardiac involvement secondary to Fabry disease. Kaplan-Meier (KM) analysis was performed for each of the ECG and ECHO parameters together with subgroup comparison of those patients with <1% residual alpha-galactosidase A activity to those with >1% residual enzyme activity. **Results:** There was no significant subgroup difference between the cardiac parameters measured and the patients genotype or the residual level of alpha-galactosidase A activity. The natural history of the ECG and ECHO parameters overlapped. 25 patients developed LVH and 2 patients developed pulmonary hypertension. One patient had dilated aortic root (6mm) and one moderate aortic stenosis. On ECG most patients fulfilled criteria for LVH, 7 patients had right bundle branch block, one patient first degree AV block and 2 patients atrial fibrillation. **Conclusions:** Progression of cardiac Fabry disease does not depend on residual enzyme activity or genotype suggesting that the heart metabolism is less tolerant to reduction of enzyme activity. Cardiac abnormalities in Fabry disease may be less amendable to treatment with enzyme replacement therapy.

Natural History of the Anderson - Fabry Disease related Cardiomyopathy. *F.A. Baehner¹, C.M. Wiethoff¹, C. Whybra², E. Miebach², M. Beck², C. Kampmann¹.* 1) Pediatric Cardiology, University Children's Hospital, Mainz, Germany; 2) Lysosomal Storage Diseases, University Children's Hospital, Mainz, Germany.

Background: Anderson - Fabry disease (AFD), an X-linked metabolic disorder, causes a progressive accumulation of globotriaosylceramide (Gb3), in a wide variety of tissues, including the development of left ventricular (LV) hypertrophy. We aimed to evaluate onset and progression of the AFD related cardiomyopathy. **Methods and Results:** 119 genetically proven hemi- and heterozygotes aged 0.5 to 70.8 years were echocardiographically evaluated. For both genders, there was a strong correlation between age and LV mass ($F < 38.12$; $P < 0.0001$). All 30 males older than 28 and all 37 females older than 38 years had abnormal LV geometry (wall thickening with small cavity) (3.4% of males, 24.3% of females) or increased LV mass (96.6% of males, 75.7% of females). Mean LV mass indexed to height (normal: $< 50 \text{ g/m}^{2.7}$) in males > 28 yrs was $78 \pm 22 \text{ g/m}^{2.7}$ and in females > 38 yrs $86 \pm 40 \text{ g/m}^{2.7}$. Most common findings were concentric hypertrophy, followed by cardiac remodelling and eccentric hypertrophy. Kaplan Meier estimation of freedom from developing severe cardiomyopathy (mean LV wall thickness ≥ 15 mm) was 39.3 years in males and 50.9 years in females. Repetitive follow up examinations of 35 pts (17 females and 18 males) revealed an increase in mean ventricular wall thickness of 0.98 mm per year. Severe Cardiomyopathy was accompanied by disturbed diastolic and systolic LV function, resulting in reduced cardiac output, and increase in NYHA heart failure functional class. **Interpretation:** The classical Anderson Fabry disease related hypertrophic cardiomyopathy affects both genders equally in terms of severity, but develops at different ages. Female heterozygotes are affected about 10 years later. **Conclusion:** Hypertrophic cardiomyopathy is a common feature in AFD. Close follow up is warranted for both genders, whether hemi- or heterozygous for AFD. This data supports the concept that AFD has X-linked dominant rather than recessive transmission.

Prolidase Deficiency: a molecular, biochemical and morphological study on five cases to investigate the molecular bases of the disease. A. Forlino¹, A. Lupi¹, P. Vaghi², A. Icaro Cornaglia³, E. Campari¹, A. Calligaro³, G. Cetta¹. 1) Dipartimento di Biochimica, University of Pavia, Pavia, Italy; 2) Centro Grandi Strumenti, University of Pavia, Pavia, Italy; 3) Dipartimento di Medicina Sperimentale, University of Pavia, Pavia, Italy.

Mutations in the prolidase gene cause Prolidase Deficiency (PD), a rare recessive disorder characterized mainly by skin lesions, mental retardation and recurrent infections. The mechanisms leading to the phenotype in PD are still poorly understood. We characterized the molecular defect in five PD patients. Direct sequencing of PCR amplified genomic DNA showed a G→A transversion in two siblings leading to a G448R substitution. A G→C transition in one allele of intron 11, causing the skipping of exon 11, was detected in a third proband and this is the first report of a mutation in a splicing donor site of the prolidase gene. A G→A transversion in intron 7 was identified in two unrelated probands and shown to cause multiple alternative spliced transcripts. Long term cultured fibroblasts from these PD patients were used to investigate the biochemical, morphological and ultrastructural changes in affected cells. By light and electron microscopy patients cells appeared swelling with increased cytosolic vacuolation, plasma membrane interruptions, mitochondria swelling and mitochondrial matrix and cristae modifications. The cellular death was evaluated by trypan blue uptake. The percentage of nonviable cells was statistically higher for the mutant than for control cells. JC-1 labelling showed decreased mitochondrial membrane potential. A significant intracellular accumulation of the Gly-Pro dipeptide was revealed by capillary electrophoresis analysis. The composite data provide new insights into PD pathophysiology, suggesting the activation in skin fibroblasts of a necrosis-like cellular death, which could be responsible for the typical skin lesions in this disease.

Molecular analysis and in vitro expression of two novel mutations identified in Italian patients affected by carbonic anhydrase II deficiency. *G. Bonapace*¹, *G. Weber*², *P. Strisciuglio*¹. 1) Dept of Pediatrics, Univ "Magna Graecia" of Catanzaro, Catanzaro, Italy; 2) Dept of Pediatrics University of Milan.

Deficiency of Carbonic anhydrase II (CAII), results in a rare syndrome characterized by osteopetrosis, renal tubular acidosis and cerebral calcification, inherited as an autosomal recessive trait. Previously (1) we reported in three Italian patients, two novel mutations: the IVS6nt1, associated to a mild phenotype without mental retardation, and Del 164 C associated to mental retardation, cerebral calcification and frequent fractures. We here describe two novel mutations recently identified. The first was a Gt insertion at the codon 10 in exon 6 of CAII gene (601insgt-stop) present in two brothers coming from Naples, characterized by absence of enzymatic activity, the second was a transition G-A at nucleotide 352 in exon 3 (352G-A stop) present in the proband coming from Lombardia, northern Italy associated to a quite residual enzymatic activity. The last mutation was confirmed by restriction analysis for the appearance of a new BfaI site. The expression in E Coli BL21DE, of two of the human cDNA containing the novel mutations, revealed for 352G-A stop mutation, absence of activity and a minimal residual activity for 601insgt stop mutation, according with their location. In fact while the 352G-A mutation introduces an early stop codon into exon 3, altering the enzymes catalytic domain, the 601 insgt stop mutation, resides in exon 6, allowing a more complete protein translation that could account for the minimal residual activity. Strikingly the expression data seem not in agree with the clinical findings of our patients having the 352G-A stop mutation a phenotype milder than the 602insgt stop mutation. We believe the minimal residual activity observed in our patient, could reflect some postranslational modification not obviously valuable in E. Coli expression system. In conclusion our data confirm the molecular heterogeneity of CAII deficiency in Italy, and suggest that in this syndrome, also the postranslational modification have to be evaluated to perform a correct genotype-phenotype correlation 1) P.Hu. et al Hum. Mut. 9,383,1997 This work was supported by COFIN MURST 1998.

Assessment of Antioxidant Status in Wilson disease children:Effect of Vitamin E and Zinc supplementation. *G. Kaur¹, R. Prasad², B.R. Thapa³, S. Kumar²*. 1) Dept of Physiology, Prayas Building,Sec-38, GMCH, Chandigarh, India; 2) Dept of Biochemistry,PGIMER, Sector-12,Chandigarh-India; 3) Dept. Of Gastroenterology,PGIMER, Sector-12, Chandigarh-India.

Wilson disease is an autosomal recessive disorder of copper metabolism. However, copper is an essential component of many enzymes, it is only required in trace quantities and is toxic in large amount due to production of hydroxyl free radicals by Fenton type of mechanism. Therefore in the present study antioxidant status was assessed by measuring antioxidant enzymes and lipid peroxidation. The supplementation of vitamin E and Zinc was also evaluated. The antioxidant enzymes like catalase, SOD, Glutathione peroxidase activities were significantly diminished in Wilson disease patient as compared to normal children of the matched age group. GSH which is a prominent scavenger of copper was significantly reduced in Wilson disease patients. Decrease in antioxidant enzymes and GSH, resulted in increase lipid peroxidation of erythrocyte membrane which was assessed by measuring MDA. Interestingly supplementation to Wilson disease patients of Vitamin E and Zinc for three months restored the antioxidant status, which was again assessed by measuring antioxidant enzymes and lipid peroxidation. In conclusion, copper influences the anti oxidant status in Wilson disease, which is normalised by supplementation of Vitamin E and Zinc.

GenotypePhenotype Analysis of American Patients with Congenital Disorders of Glycosylation-IA (CDG-1A). *D. Krasnewich¹, E. Orvisky², M. LaMarca², D. Andrews², N. Tayebi², O. Goker-Alpan², E. Sidransky².* 1) Medical Genetics Branch, NHGRI/NIH, Bethesda, MD; 2) Section of Molecular Neurogenetics, NIMH/NIH, Bethesda, MD.

CDG are a group of metabolic disorders resulting from the defective synthesis of N-linked glycans. Eight types of CDG have been defined with distinct enzyme defects and genes; the most common is CDG-1A. CDG-1A is caused by the deficiency of phosphomannomutase (PMM) resulting from mutations in the gene, PMM2. The genotypes and phenotypes of 23 patients with CDG-1A, including 3 sib pairs were studied. 90% of the mutant alleles were identified including 14 different mutations in 20 probands. As expected from other reports, R141H/F119L was the most common genotype; no homozygous genotypes were identified. PMM was assayed in lymphoblasts or fibroblasts from patients by HPLC. PMM enzyme activities ranged from 0-18% of control. Children are most commonly ascertained during the workup for failure to thrive (FTT), developmental delay, elevated liver transaminases, coagulopathy or cerebellar hypoplasia. Children with the most severe clinical presentations of CDG-1A (3/23) die in infancy from medical sequelae of FTT, hypoalbuminemia and intractable fluid imbalance. Other patients have a milder course of developmental delay, ataxia and slow growth. However, when comparing the clinical course of these subjects there was no apparent correlation between enzyme activity, genotype and phenotype. For example, 2 children sharing genotype, R141H/V231M, had different PMM activities and dramatically different clinical courses. The first child, with less than 1% of control enzyme activity, died in infancy while the second, with 13% of control, had a more typical course with hypotonia, FTT and seizures. Similarly, 2 affected individuals with genotype R141H/F119L also had discordant phenotypes. Surprisingly, the child with higher PMM activity, 5% of control values, had a catastrophic newborn course, while the child with 1% of control activity had moderate developmental delay and FTT. Clearly, other factors, including environmental and genetic modifiers, may contribute to the clinical phenotype in this complex disorder.

Growth Retardation in Children and Adolescents with Type B Niemann-Pick Disease. *M.P. Wasserstein, A.E. Larkin, R.B. Glass, R.J. Desnick, E.H. Schuchman, M.M. McGovern.* Mount Sinai School of Medicine, NY, NY.

Types A and B Niemann-Pick disease (NPD) are rare inherited lysosomal storage disorders caused by deficient activity of acid sphingomyelinase. Type A NPD is characterized by failure to thrive, severe neurologic disease, hepatosplenomegaly, and death in infancy. In contrast, patients with type B NPD have multi-organ involvement, including hepatosplenomegaly, hyperlipidemia, and progressive infiltrative pulmonary disease with sparing of the nervous system. The phenotypic spectrum of type B NPD ranges from severe disease with death in childhood to a milder disease in adults with hepatosplenomegaly. Although failure to thrive is a recognized feature of type A NPD, growth parameters have not been well described in children with type B NPD. We therefore performed a cross-sectional analysis of growth in 23 children and adolescents with type B NPD. Anthropometric parameters were compared with bone age, organomegaly, serum levels of insulin-like growth factor-1 (IGF-1), and genotype. Height was at or below the 5th percentile in 39% of all subjects. The mean z score for height was 1.24, corresponding to the 29th percentile. Weight was at or below the 5th percentile in 21% of subjects. The mean z score for weight was -0.75, corresponding to the 34th percentile. The mean bone age delay, measured as bone age minus chronological age, was 2.57 years. The mean liver volume was 2.07 times normal for weight, and the mean spleen volume was 14 times normal for weight. Serum IGF-1 was below the 2nd percentile for age and sex in 7 of the 10 patients in whom it was determined, and correlated with short stature. When considering genotype, striking differences were found between patients who were homozygous for the most common allele, delR608, vs. patients with all other genotypes. The delR608 homozygotes were of normal height and weight, had less organomegaly, less bone age delay, and normal serum IGF-1 levels. These findings suggest that 1) abnormal linear growth associated with delayed skeletal maturity is common in children and adolescents with type B NPD, and 2) homozygosity for the delR608 allele may be associated with a milder phenotype.

Biotin availability regulates the expression of enzymes involved in its utilization and recycling in human cells. *D. Pacheco-Alvarez*¹, *S. Solorzano-Vargas*¹, *A. Velazquez*², *J. Zemleni*³, *A. Leon-Del-Rio*¹. 1) Department of Molecular Biology, UNAM, Mexico D.F., Mexico, D.F., Mexico; 2) Department of Genomic Medicine and Toxicology; 3) Department of Nutritional Genetics, University of Nebraska-Lincoln.

In human cells biotin is used as cofactor of enzymes involved in carboxylation reactions. Biotinylation of these enzymes is catalyzed by holocarboxylase synthetase (HCS). Recently, we showed that biotin and HCS are required to maintain the mRNA levels of HCS and of biotin-dependent carboxylases genes in human cells. In this work we show the effect of biotin availability on the mRNA levels of the sodium dependent multivitamin transporter (SMVT) which is responsible for the vitamin uptake in humans. We present evidence of a correlation between SMVT expression and the mRNA levels of enzymes involved in biotin utilization in human cells. Our results showed that biotin deficiency in human hepatocytes and fibroblasts results in a 70 % reduction on both the mRNA levels and protein amount of SMVT. Stimulation of biotin deficient cells with biotin or cGMP restored the SMVT mRNA levels to within normal values after 24 hr. Similar effects on mRNA levels were observed for the genes encoding HCS and pyruvate carboxylase (PC). We suggest that biotin deficiency results in the downregulation of the expression of enzymes such as HCS, PC and SMVT in peripheral tissues to allow a continuous supply of biotin to the brain. This hypothesis is confirmed by studying the expression of all these genes in an animal model for biotin deficiency. In these animals, biotin deficiency resulted in a decrease in the mRNA levels of HCS, SMVT and PC in liver, kidney and muscle. However, mRNA levels of all these enzymes were not affected in brain by biotin deficiency. The mechanism for biotin utilization reported here is impaired in the disease multiple carboxylase deficiency and this may cause some of the symptoms observed in patients. The mechanism proposed for biotin utilization is very similar to the well known mechanism of glucose utilization in muscle and brain during fasting.

Insights into obesity and glucose homeostasis from study of Angelman syndrome mouse models. *R.D. Nicholls¹, Y. Qi¹, D.J. Driscoll², M. Dhar³, R.S. Ahima¹*. 1) University of Pennsylvania, Philadelphia, PA; 2) University of Florida, Gainesville, FL; 3) University of Tennessee, Knoxville, TN.

Angelman syndrome (AS) patients have genetic defects in chromosome 15q11-q13, with neurological disease due to loss of maternal-only imprinted expression of the *UBE3A* gene in some brain regions. AS subjects in several molecular subclasses have obesity, with a body mass index in the 80th-100th percentile for age. Similarly, an AS mouse model with a chromosome deletion and mice with maternally-inherited, radiation-induced deletions of the *Atp10c* gene have obesity by 4-6 months (mo) of age. We have performed physiological and metabolic studies of AS mice to gain insight into the pathophysiological basis of obesity in AS. When AS (CD-1) deletion mice fed a standard low (4%) fat chow diet were placed on a high (45%) fat diet, within 4 weeks (wks) they became obese compared to similarly treated wildtype (WT) littermates [12 wks, AS=31.1+/-0.4 g vs. WT=29.3+/-1.8 g, p=0.506; 20 wks, AS=46.8+/-1.9 g vs. WT=35.5+/-1.5 g, p=0.012]. AS mice of normal weight at 3 mo show mild glucose intolerance, with a glucose level 30 min after an i.p. injection of 20% glucose (2g/kg) of 196+/-2.3 mg/dl vs. WT (156+/-12.7 mg/dl; p=0.036). After a 4 wk high fat diet, AS mice have significant glucose intolerance (30 min, AS=423.5+/-25.2 mg/dl vs. WT=261+/-33 mg/dl, p=0.096; 60 min, AS=221+/-14.9 mg/dl vs. WT=174.5+/-7.5 mg/dl, p=0.041). The weight gain in AS mice on a high fat diet was not dependent on increased food intake (AS=2.5+/-0.1 g vs. WT=2.5+/-0 g, p=0.678); in contrast, indirect calorimetry revealed a lower oxygen consumption (AS=2051+/-50 ml/kg/h vs. WT=2365+/-71 ml/kg/h, p=0.003). Therefore, the loss of *Atp10c* function may predispose to obesity by lowering energy expenditure. Similar studies of AS and WT mice on several genetic backgrounds are ongoing to give insight into the natural history of glucose impairment in this mouse model. Studies are also underway on *Atp10c* imprinting, expression and function, putatively in some aspect of lipid translocation. This work will further an understanding of the genetic control of glucose homeostasis and obesity in animal models and the human population.

Energy metabolism and glucose homeostasis in a mouse model of Prader-Willi syndrome. *M. Stefan*¹, *H. Ji*², *S. Frayo*³, *H.R. Patel*¹, *D.E. Cummings*³, *R.S. Ahima*¹, *M.I. Friedman*², *R.D. Nicholls*¹. 1) University of Pennsylvania, Philadelphia, PA; 2) Monell Chemical Senses Center, Philadelphia, PA; 3) University of Washington, Seattle, WA.

Prader-Willi syndrome (PWS) subjects have neonatal failure-to-thrive and hypotonia, with hyperphagia and obesity starting in early childhood, short stature, abnormal fat and muscle composition, hypogonadism and abnormal behavior. PWS is caused by loss of function of several paternally expressed, imprinted genes in a 1.5 Mb region of chromosome 15q11-q13. Candidate genes include 5 protein-coding genes and 5 classes of snoRNAs, many of which appear to have regulatory function and may regulate genetic pathways leading to the clinical phenotype (e.g., GH and IGF1 deficiency). Mouse models of PWS recapitulate the neonatal phenotype with severe failure-to-thrive and survival for 1 week only. The basis of PWS is not known, and we show here that a mouse model has a widespread metabolic dysfunction. Abnormalities in PWS *vs.* wildtype (WT) pups include severe hypoglycemia, low insulin levels and high liver glycogen. Oxygen consumption and heat, measured by indirect calorimetry, were markedly reduced in PWS mice. Although there were no differences in liver ATP for postnatal day 1 (P1) between PWS and WT mice (0.990 +/- 0.66 *vs.* 0.89 +/- 0.55 umol/g; n=5-7; p=0.18), liver ATP was significantly higher in P2 PWS mice (1.34 +/- 0.10 *vs.* WT, 0.84 +/- 0.003 umol/g; n=5-8; p<0.0001) as well as for P3-P4 pups. Liver and blood ketones were significantly higher in P1-P4 PWS *vs.* WT mice while circulating free fatty acids and triglycerides were lower. PWS (P1) mice had higher plasma ghrelin levels than WT mice (2919 *vs.* 2316 pg/ml, n = 15-17, p=0.017), suggesting abnormal regulation of this orexigenic hormone. Our data suggest that PWS mice have abnormal energy metabolism, possibly caused by accelerated fat oxidation despite severe deficits in glucose and fat fuel supplies. Additional studies are ongoing with microarray technology to examine the global gene expression patterns in liver and brain of PWS and WT mice. This approach will define genetic pathways leading from the imprinted genes to the metabolic changes in PWS.

Adenine phosphoribosyltransferase deficiency and recurrent nephrolithiasis. *L. Deng¹, L. Liang¹, R. Chiarle², P. Barocelli³, P.F. Celle², C. Salerno⁴, H.A. Simmonds⁵, L. Fairbanks⁵, J.A. Tischfield¹, A. Sahota¹.* 1) Dept. of Genetics, Rutgers Univ., Piscataway, NJ; 2) Dept. Anatomic Pathology, Univ. Turin, Italy; 3) Pediatric Unit, Chivasso Hospital, Italy; 4) Clinical Biochem., La Sapienza Univ., Rome, Italy; 5) Purine Research Unit, Guys Hospital, London, UK.

Adenine phosphoribosyltransferase (APRT) deficiency results in 2,8-dihydroxyadenine (DHA) nephrolithiasis and in some cases renal failure. We examined the mutational basis of APRT deficiency in a 45-year-old patient of Italian origin who passed crystals and stones, initially thought to be made-up of urate, between ages 4-7. The left kidney was described as hydronephrotic but the right kidney was normal. Radioisotope studies at age 14 showed severely impaired renal function, but follow-up studies at age 34 did not demonstrate significant changes. A large stone (identified as >90% DHA) was present in the left kidney but the right kidney was again normal. The patient was placed on a low purine diet and high water intake. He remained asymptomatic until age 44, when he developed anemia and polyuria, and a stone was present in the right kidney. The patient had no detectable APRT activity in erythrocytes, and low levels of DHA were present in the urine and serum. The five exons and flanking regions of APRT were amplified by PCR and sequenced. A novel A-to-G transition was identified at the intron 2 splice acceptor site (AG-to-GG). The parents are first cousins and they were both heterozygous for the mutation, confirming that the patient is homozygous deficient. This was verified by Stu I digestion of PCR-amplified DNA spanning exon 3. The transition alters the reading frame downstream of codon 62 and is expected to abolish enzyme activity. The patient is on allopurinol and dialysis, waiting for a transplant. APRT deficiency was discovered nearly thirty years ago, and a variety of methods are available for its rapid diagnosis. However, as this case illustrates, APRT deficiency continues to be unrecognized or untreated, possibly due to the asymptomatic nature of the disease and the long intervals between stone episodes in some patients. Supported by NIH grant DK 38185.

Sibling phenotype concordance in classical infantile pompe disease. *W.E. Smith*¹, *J.A. Sullivan*², *P.S. Kishnani*², *Y-T. Chen*², *D. Corzo*³, *G.F. Cox*³. 1) Barbara Bush Children's Hospital, Maine Medical Center, Portland, ME; 2) Duke University Medical Center, Durham, NC; 3) Genzyme Corporation, Cambridge, MA.

Background: Glycogen storage disease, type II, includes severe (classical infantile, Pompe disease), moderate (non-typical infantile and juvenile), and mild (adult) forms that generally correlate with the degree of acid--glucosidase deficiency. Sibling phenotype discordance has been reported for later-onset forms of glycogen storage disease, type II, but has not been studied in classical infantile disease.

Methods: The literature was reviewed for affected sibships in which at least one sibling (the proband) had clinical and either histologic or biochemical features consistent with classical infantile Pompe disease. Age of onset, age of death, and clinical course were compared between probands and affected siblings.

Results: From 1930 to 1986, 14 case reports described 12 such families with a total of 29 affected infants. There were 10 probands and 19 affected siblings. The average age at onset of symptoms for all affected infants was 2.7 months (STD 1.5), 2.4 months (STD 1.4) for probands, and 3.0 months (STD 1.6) for siblings. The average age at death for all affected infants was 5.6 months (STD 2.6), 6.8 months (STD 3.1) for probands, and 5.0 months (STD 2.2) for siblings. The average length of disease course for all affected infants was 3.3 months (STD 2.5), 4.5 months (STD 2.9) for probands, and 2.5 months (STD 1.8) for siblings. Probands and siblings showed similar ages of onset and death, clinical symptoms, and causes of death that reflect the characteristic cardiac and respiratory compromise.

Conclusions: Unlike later-onset forms of glycogen storage disease, type II, there appears to be little to no phenotypic variation, especially in terms of lifespan, among siblings with classical infantile Pompe disease.

Short Tandem Repeat Polymorphism at Phenylalanine Hydroxylase Locus and its Application to Prenatal Diagnosis and Carrier Detection of Phenylketonuria in the Iranian Population. *N. Hoseinpur, Kh. Anisi, S.M. Seifati, Z. Khazae-Koohpar, S.M. Hosseini-Mazinani.* Medical Genetics, National Research Center for Genetic Engineering and Biotechnology, Tehran, Iran.

A polymorphic short tandem repeat (STR) marker at phenylalanine hydroxylase locus shows an informativity of 80 percent in prenatal diagnosis and carrier detection of phenylketonuria (PKU) in European PKU kindreds and 73 percent in Chinese PKU kindreds. To investigate the usefulness of this marker in carrier detection and prenatal diagnosis of phenylketonuria in Iranian population, allelic distribution of STR polymorphism was studied in 10 Iranian PKU families and segregation analysis of this polymorphism was performed for 6 siblings of PKU patients. The STR fragments were amplified by PCR and alleles were separated by acrylamide gel electrophoresis. In 8 families (80% of the families), distribution of STR alleles was deemed to be informative. Segregation analysis of STR alleles yielded informative results in all 6 cases and the carrier status could be determined. Our findings indicate a high level of informativity for this marker in carrier detection and prenatal diagnosis in Iranian PKU families. Our results are comparable to those obtained in European Caucasian and Oriental populations.

The Association of Gaucher Disease and Parkinsonism. *J.M. Walker¹, N. Tayebi¹, M. Hanson², B.K. Stubblefield¹, E. Sidransky¹.* 1) Sec on Molecular Neurogenetics, National Institute of Mental Health, Bethesda, MD; 2) Lab of Neurogenetics, NIA.

Gaucher disease, the inherited deficiency of glucocerebrosidase, presents with many diverse phenotypes including a rare group of patients who develop parkinsonian manifestations. While the basis for this association is unknown, patients with this atypical Gaucher phenotype often have early onset, treatment refractory parkinsonism but few Gaucher related symptoms. Mutation analysis of fifteen such patients revealed no shared genotype, although many carried at least one allele with mutation N370S. No mutations in the genes for alpha synuclein or parkin were identified. Of note, several of the probands had family members with parkinsonism but without Gaucher disease. Post mortem studies performed on one of the patients demonstrated diffuse Lewy body inclusions in the hippocampus, substantia nigra, pyramidal neurons and midbrain structures, but not in the cortex. The concurrence of Gaucher disease and parkinsonism, both in these patients and in others in the literature and the unusual neuropathological findings suggest a shared pathway, modifier or other genetic etiology for the two phenotypes. To further explore this association, the gene for glucocerebrosidase is being examined in a large series of probands with early-onset Parkinson disease, but without known Gaucher disease. Exons 8-11 of glucocerebrosidase have been sequenced in 65 individuals and two alterations, E326K and T369M have been identified, on each allele. Studies of this association may yield valuable insights into the neuropathophysiology of both Gaucher disease and parkinsonism.

Identification of the mouse buff (*bf*) gene, a candidate for human Hermansky-Pudlak syndrome (HPS). *N. Oiso*¹, *T. Suzuki*¹, *E.K. Novak*², *R.T. Swank*², *R.A. Spritz*¹. 1) Human Med Genetics, Univ Colorado HSC, Denver, CO; 2) Mol Cell Biol, Roswell Park Canc Inst, Buffalo, NY.

Hermansky-Pudlak syndrome (HPS) is an autosomal recessive disorder characterized by albinism, bleeding, and lysosomal storage, with frequent fatal complications. In mouse, more than 15 loci are associated with mutant phenotypes similar to human HPS. We have mapped and positionally cloned one of these loci, buff (*bf*), characterized by reduced coat coloration and hypopigmented melanocytes with a reduced number of melanized melanosomes, and by reduced platelet aggregation.

The mouse *bf* locus is located at 64 cM on chromosome 5. Within the *bf* genetic interval we identified a strong biological positional candidate, *Vps33a*, a mammalian homologue to the yeast vacuolar protein sorting (Vps) mutant *Vps33*. *Vps33* belongs to the yeast class C Vps protein complex, composed of Vps11, Vps16 and Vps18 and involved in the regulation of vacuolar SNARE pairing and vesicle docking/fusion.

DNA sequence analysis of *Vps33a* in *bf*-mutant mice identified a missense substitution, D251E (GAT®GAG), not present in the C57BL/6J background strain. The D251 residue has been absolutely conserved throughout evolution, strongly suggesting that the D251E substitution is pathologic. We recapitulated the D251E mutation in mouse *Vps33a* cDNA, and stably transformed *bf*-mutant melanocytes, whose abnormal melanosomal phenotype was complemented by stable transformation with wild-type *Vps33a* cDNA, but not by the D251E cDNA or by empty vector. These results confirm that the D251E substitution is pathologic, and that *Vps33a* is thus the murine *bf* locus.

To determine whether human *VPS33A* is a major HPS locus, we screened for mutations in 24 HPS patients who lacked mutations in the *HPS1*, *HPS2*, *HPS3*, *HPS4*, or another HPS gene. One patient was heterozygous for a novel substitution, I256L; however, we did not find any other mutation in this patient and conclude that this substitution is a rare polymorphism.

Fabry Disease: Unrecognized ESRD Patients and Effectiveness of Enzyme Replacement on Renal Pathology and Function. *R.J. Desnick.* Dept Human Genetics, Department of Human Genetics, Mount Sinai School Medicine, New York, NY.

Classical Fabry disease (FD), α -galactosidase A deficiency (α -gal A), is an X-linked lysosomal storage disease that leads to end-stage renal disease (ESRD) in the third to fifth decades of life. In affected males, the progressive accumulation of glycosphingolipid substrates, predominantly globotriaosylceramide (GL-3), in the plasma and in lysosomes of blood vessels and other tissues leads to onset of angiokeratoma, hypohydrosis, acroparesthesias, corneal dystrophy in childhood with vascular disease of the heart, brain, and kidney (ESRD) causing early demise in adulthood. Recently, enzyme replacement therapy (ERT) was shown to be safe and efficacious in a double-blind randomized control Phase 3 study (*New Engl. J. Med.* 345: 9-16, 2001). Results of the Phase 3 extension trial demonstrated GL-3 clearance in renal endothelial, mesangial and interstitial cells with reduced GL-3 in podocytes and tubular epithelium. These findings emphasize the importance of identifying underdiagnosed affected males with ESRD, since later complications include cardiac and cerebro-vascular disease. To investigate the possibility that unrecognized ESRD patients undergoing chronic dialysis have FD, independent studies were conducted in Japanese, Italian, and American centers to identify such patients by screening for deficient α -gal A in plasma and/or leukocytes. In Japan, 6 of 514 males (1.2%), in Italy 18 of 1522 males (1.2%), and in the United States, 9 of 1903 (2.1%) males on dialysis (typically diagnosed chronic glomerular sclerosis) had FD. These ESRD patients lacked angiokeratoma, acroparesthesias, hypohydrosis, and/or corneal changes, precluding their earlier diagnosis of FD. These studies indicate that 1-2% of ESRD patients may be undiagnosed FD renal variants who would benefit from ERT, which can be accomplished during dialysis.

Fabry disease in Europe: update from FOS — Fabry Outcome Survey. A. Mehta¹, M. Beck², J. Ahlmen³, T. Cox⁴, F. Dehout⁵, J-O. Johansson³, A. García de Lorenzo⁶, K. MacDermot¹, R. Ricci⁷, M. Ries², G. Sunder-Plassmann⁸, U. Widmer⁹. 1) Royal Free Hospital, London, UK; 2) University of Mainz, Mainz, Germany; 3) Sahlgrenska University Hospital, Göteborg, Sweden; 4) University of Cambridge, Cambridge, UK; 5) CHU, Charleroi, Belgium; 6) Formación Médica Continuada Hospital Universitario La Paz, Madrid, Spain; 7) Università Cattolica del Sacro Cuore, Rome, Italy; 8) University of Vienna, Vienna, Austria; 9) University Hospital, Zurich, Switzerland.

Introduction: Fabry disease is a lysosomal storage disorder due to deficiency of the enzyme α -galactosidase A. Enzyme replacement therapy with agalsidase alfa has recently been shown to have significant clinical benefits. FOS—Fabry Outcome Survey—has therefore been established to follow the long-term effects of treatment, and currently contains data on nearly 200 patients (>33% female).

Methods: Data on the baseline characteristics of patients in FOS were analysed.

Results: Age at entry into FOS ranged from 9 months to 71 years. For males the diagnosis of Fabry disease was most frequently first suspected by nephrologists or dermatologists, whereas affected family members were most likely to be the first to suspect Fabry disease in females. Misdiagnosis was common, and the mean age of diagnosis was 25 years in males and 37 in females. The most common signs and symptoms in both sexes were neurological, cardiac and dermatological, and the number of organs affected increased with age. No patient had only cardiac involvement. Proteinuria was present in 52 and 39% of males and females, respectively. Mean age at start of agalsidase alfa treatment was 35 and 44 years in males and females, respectively.

Conclusions: There is a clear need for increased awareness of Fabry disease, to allow early diagnosis and treatment. It is also clear that many heterozygous women are affected by the disease.

Coenzyme Q-responsive Leigh encephalopathy in two sisters. *I. Maystadt¹, F. Trijbels², S. DiMauro³, P.J. Sindelar⁴, O. Musumeci³, A. Janssen², X. Delberghe⁵, J.J. Martin⁶, L. Van Maldergem¹.* 1) Ctr Human Genetics, Inst Pathology and Genetics, Loverval, Belgium; 2) Ctr Mitochondrial Disorders, Clinical Genetics Ctr, University of Nijmegen, The Netherlands; 3) Columbia University, College of Physicians and Surgeons, Dpt of Neurology, New York, USA; 4) Dpt Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden; 5) Institut Medicopedagogique Montfort, Herseaux, Belgium; 6) Dpt of Neuropathology, Born-Bunge Foundation, University of Antwerp, Antwerp, Belgium.

A 31-year-old woman had encephalopathy, growth retardation, infantilism, ataxia, deafness, lactic acidosis and increased signals of caudate and putamen on brain MRI. Muscle biochemistry showed succinate:cytochrome c oxidoreductase (complex II-III) deficiency. Both clinical and biochemical abnormalities improved remarkably with CoQ10 supplementation. Clinically, on 300 mg CoQ10/day, she resumed walking, gained weight, underwent puberty, and grew 20 centimeters between 24 and 29 years of age. CoQ10 was markedly decreased in CSF, muscle, lymphoblasts, and fibroblasts, suggesting the diagnosis of primary CoQ10 deficiency. An older sister has similar clinical course and biochemical abnormalities. These findings suggest that CoQ10 deficiency can present as adult Leigh syndrome.

Lessons learned from an attempt to rescue the phenotype in acid alpha-glucosidase knockout mice. *N. Raben, T. Jatkar, A. Lee, P. Plotz.* NIAMS, ARB, NIH, Bethesda, MD.

Deficiency of acid alpha-glucosidase (GSDII, Pompe disease) results in generalized deposition of lysosomal glycogen and manifests as myopathy and fatal cardiomyopathy. A knockout mouse model (GAA^{-/-}) resembles the most severe human infantile form of the disease. To rescue the phenotype of ^{-/-} mice, we have made transgenic lines expressing different levels of human GAA cDNA (hGAA) in skeletal muscle or liver of these mice by using a tetracycline-controllable system. The system allowed us to demonstrate the advantage of liver-mediated gene transfer for secretion and systemic delivery of the enzyme and address the issue of reversibility of the disease by turning the transgene on at different stages of the disease progression. Although many lysosomal disorders are corrected by 1-2% of normal level of enzyme activity, it has been generally accepted that 20-30% of normal GAA activity, provided by gene or enzyme replacement therapy, would be required to reverse the myopathy and cardiomyopathy in Pompe disease. We have demonstrated that the levels of 20-30% of normal enzyme activity are indeed sufficient to clear glycogen in cardiac muscle of young (2 months of age) ^{-/-} mice, but not in older (6 months of age) mice with advanced pathology and a considerably higher glycogen load. Thus, at later stages of disease progression, the required therapeutic level of enzyme activity may be much higher than previously expected. Skeletal muscle a major organ affected in both infantile and late-onset forms without cardiac involvement has proved to be a much greater challenge. By contrast with the outcome in cardiac muscle, the levels of 22-38% wild type were far from sufficient to clear glycogen completely in skeletal muscle resulting in only 43-46% glycogen reduction. Furthermore, induction of hGAA expression in young ^{-/-} mice to the levels greatly exceeding wild type values did not result in full phenotypic correction, and some muscle fibers showed little or no glycogen clearance. The results emphasize the importance of early intervention and point to problems ahead in the first attempts to treat a muscle disorder with enzyme replacement therapy.

A Nova Scotia (NS) Kindred with Fabry Disease (FD). *M. West*¹, *S. Dyack*², *C. Riddell*³, *K. Lemoine*¹, *C. Camfield*⁴, *P. Camfield*⁴. 1) Departments of Medicine; 2) Medical Genetics; 3) Pathology; 4) Pediatrics, Dalhousie University, Halifax, Nova Scotia, Canada.

We describe a kindred in NS with classic FD and a uniform genotype. All records for all FD patients in NS were retrospectively reviewed. This kindred of 948 in 14 generations (100 heterozygotes and 65 hemizygotes) arose with a woman of German parentage born in 1799 on Tancook Island off the southeast coast of NS. Diagnosis of FD was based on a-galactosidase A activity, DNA mutational analysis and clinical features. All NS FD patients are of this kindred based on pedigree and/or DNA mutational analysis with a missense mutation A143P in exon 3. Almost all live within 100 miles of Tancook Island consistent with a founder effect. There are 4 smaller kindreds of Fabry disease in NS of 72 individuals, including 9 hemizygotes and 18 heterozygotes. While unrelated by pedigree to the largest kindred of 876, the 4 smaller kindreds are uniform in terms of classic phenotype, A143P mutation and geographic distribution. In 2000 there were 36 heterozygotes and 27 hemizygotes alive, total 63, from all 5 NS kindreds. Three males (ages 25-48 years) are alive with renal transplants; two have cardiomyopathy and cerebrovascular disease. Another male (age 48 years) is on hemodialysis awaiting a renal transplant. All four are receiving enzyme replacement therapy (ERT) with a-galactosidase A. Another 7 males and 1 female are enrolled in clinical trials of ERT. ERT was well tolerated by all. The details of this kindred will be displayed. This is one of the largest FD kindreds in the world and is an important resource for ongoing study because of its size and uniform genotype.

Osteopontin and kidney stone disease. *H.J. Tinkel-Vernon¹, C. Osborne¹, H.A. Simmonds², M. Yang¹, B.J. Zerlanko¹, S. Rittling¹, D.T. Denhardt³, A. Sahota¹, J.A. Tischfield¹.* 1) Dept Genetics, Rutgers Univ, Piscataway, NJ; 2) Purine Research Unit, Guy's Hospital, London, UK; 3) Dept of Cell Biology and Neuroscience, Rutgers Univ, Piscataway, NJ.

Osteopontin (OPN) has multiple functions in the kidney, including inhibition of stone formation and propagation of inflammation via macrophage attraction. We created *Opn/Aprt* double knockout mice to delineate the role of OPN in renal stone disease. *Aprt*^{-/-} mice develop 2,8-dihydroxyadenine (DHA) nephrolithiasis similarly to patients with adenine phosphoribosyltransferase (APRT) deficiency. This is due to the conversion of adenine (A) to DHA by xanthine dehydrogenase (XDH), which converts hypoxanthine (H)/xanthine (X) to uric acid (UA). *Opn*^{-/-} mice show normal renal histology. The lack of spontaneous renal deposition of lithogenic substances in *OPN*^{-/-} mice suggests that OPN may not have a protective role *in vivo* in the absence of additional renal insult. The *Opn*^{-/-}*Aprt*^{-/-} mice allow us to study the role of OPN in renal stone disease. Pooled urine was collected from 3-4 *Aprt* and *Opn/Aprt* mice at 1-3 months old and analyzed for purines, creatinine, and other metabolites by reverse phase HPLC. The levels of total purines (TP = A, DHA, H, X, UA), oxypurines (OP = H, X, UA), and total adenines (TA = A, DHA) were normalized to creatinine (mmol/mmol). TP excretion in the *Aprt* and *Opn/Aprt* mice was comparable (0.650 v 0.648), but the *Aprt* mice excreted more TA (0.416 v 0.266). The OP/TP, TA/TP, DHA/TA ratios in the *Aprt*^{-/-} mice were 0.36, 0.64, and 0.75, respectively. The ratios in the *Opn/Aprt* mice were 0.59, 0.41, and 0.58. These data show less conversion of A to DHA in the double knockout mice, which is also reflected in the increased OP content, mainly UA. The shift towards a more normal urine purine profile in the *Opn*^{-/-}*Aprt*^{-/-} mice may be due to less macrophage infiltration, and hence less injury at the sites of DHA deposition. Release of XDH by macrophages may contribute to the conversion of A to DHA in *Aprt*^{-/-} mice. Therefore, OPN may promote injury in DHA-induced stone disease via increased inflammation. Histopathological studies are in progress. Supported by NIH grant DK38185.

Molecular characterization of a large genomic deletion including a part of the HPRT gene responsible for the Lesch-Nyhan syndrome. *Y. Yamada*¹, *N. Nomura*¹, *K. Yamada*¹, *N. Igarashi*², *N. Wakamatsu*¹. 1) Dept Genetics, Inst Dev Res, Aichi Human Service Ctr, Kasugai Aichi, Japan; 2) Toyama Centrl Hosp, Toyama, Japan.

Inherited mutations of a purine salvage enzyme, hypoxanthine guanine phosphoribosyltransferase (HPRT, MIM308000), give rise to Lesch-Nyhan syndrome (MIM 300323) or HPRT-related gout (MIM 300323). We have identified a number of such HPRT mutations in the patients manifesting different clinical phenotypes, by analyses of all nine HPRT exons from the genomic DNA and reverse transcribed mRNA using polymerase chain reaction (PCR) technique coupled with direct sequencing. In a typical Lesch-Nyhan patient deficient in HPRT mRNA, we identified a large genomic deletion (~15 kb) including the promoter region and the whole of exon 1. PCR amplification from the patient's genomic DNA showed lacking of HPRT exon 1, and RT-PCR analysis demonstrated deficiency of HPRT mRNA. In exons 2 to 9 of the gene from this patient, no mutations were found. Southern analysis using *Eco* RI and *Pvu* II digested genomic DNAs revealed a more than 13-kb deletion and we could identify by PCR methods the mutant gene deleted about 15 kb upstream from the HPRT intron 1, referring to the nucleotide sequences of the HPRT gene locus (M 26434) and a Bac clone AL590875 containing the HPRT gene. Furthermore, we carried out a family study by PCR amplification of normal and mutant genes. The mother was diagnosed as a heterozygous carrier, but the grandmother was negative and the sister had not inherited the mutant gene. Thus, the deletion mutation was a *de novo* event that had occurred either in germ cells of the grandmother or grandfather, or in the mother during the early phase of fetal development.

Molecular studies of CPS I gene: determination of genomic organization and mutation detection. *S. Funghini, M.A. Donati, E. Pasquini, E. Zammarchi, A. Morrone.* Dep. of Pediatrics, University of Florence, Florence, Italy.

Carbamyl Phosphate Synthetase I (CPS I) deficiency (CPSD) is a rare autosomal recessive urea cycle disorder usually characterized by potentially lethal neonatal hyperammonemia. Based on the age of onset, clinical symptoms and partial residual enzymatic activity, two distinct CPSD clinical phenotypes can be identified: neonatal and late-onset. The structural gene of CPS I has been mapped in the long arm of chromosome 2 at q35. The large (5215 bp) CPSI-cDNA expressed only in liver and epithelial cells of intestinal mucosa has been cloned. So far only a few genetic lesions have been described, while a lot of discrepancies have been reported between the CPSI cDNA cloned by two different groups. Up to now the CPSI genomic organization was unknown. Taking advantages of the phylogenetic lineage between the CPSI gene of *Homo sapiens* and *Rattus Norvegicus* we determine the intron-exon organization of the human CPS I gene. Starting from the ATG, the CPS I gene is organized in 38 exons spanning from 200 to 500 bp. The full-length cDNA sequences from different tissues didn't show any discrepancies, consequently any of these tissues can be used to identify the genetic lesions in the CPS I gene. We also report the molecular studies of an Italian patient affected by CPSD. Two new genetic lesions c1370T>G and c2429A>G that lead to the new V457G and Q810R amino acid substitutions were detected in the patients CPS I DNA isolated from peripheral blood lymphocytes. In addition we have detected the presence of numerous polymorphisms on the patients DNA at heterozygous level. The polymorphic discrepancies come out from the two different CPSI cDNA cloned by two different groups. These findings lead us to believe that our patient descends from two different ancestral backgrounds. The characterization of the intron-exon organization and of genetic lesions in CPSD patients will allow us to offer better genetic counselling and prenatal diagnosis.

Phenotypic characterization and functional consequences of a mutation (ORNT1-T32R) in the human mitochondrial ornithine transporter. *J.A. Camacho¹, R. Mardach², N. Rioseco¹, S. Cress², S. Cedarbaum³.* 1) Human Genetics, OUHSC, Oklahoma City, OK; 2) Medical Genetics, Kaiser Permanente, Los Angeles, CA; 3) MRRC, UCLA, Los Angeles, CA.

Human ORNT1 is the gene defective in the hyperornithinemia-hyperammonemia-homocitrullinemia (HHH) syndrome. Clinical features may include developmental delay, ataxia, coagulation abnormalities and a cognitive function that varies from low normal intelligence to severe retardation. We studied two families of Mexican descent (probands HHH13 & HHH15) that have a mild but variable phenotype. HHH13: The older sibling (18 y/o) was initially diagnosed at the age of 9 during an evaluation for poor school performance. Currently, three symptomatic brothers are in special education classes; psychometric evaluation revealed an IQ of 76 and 80 in the two youngest and 70 in the oldest. HHH15: The youngest sibling (6 y/o) was diagnosed at the age of 3 during an evaluation for gastroenteritis which demonstrated abnormal liver enzymes and coagulation studies, hypoglycemia, mild hyperammonemia and significant hyperornithinemia. His sister was subsequently diagnosed at age 13. Psychometric testing revealed an IQ of 79 in both children who are doing well in school. Analysis of the ORNT1 gene revealed a T32R mutation in both families that was not observed in 128 control alleles. We transfected cultured HHH fibroblasts with an Nmyc-tagged ORNT1-T32R construct and assayed for its ability to restore ornithine metabolism. Relative to the wild-type ORNT1 allele (3-fold baseline activity), the ORNT1-T32R allele is a decrease of function mutation (1.5-fold) with a normal mitochondrial targeting pattern. Interestingly, ORNT2 analysis demonstrated that both HHH15 siblings are heterozygous for an ORNT2-Gly181 allele that is a gain of function polymorphism when compared to the wild-type ORNT2-Val181 allele. These observations suggest that the milder phenotype of these five HHH patients may, in part, be attributed to the partial retention of ornithine transport activity exhibited by the ORNT1-T32R allele. However, ORNT2 and other unknown factors may also play a role in the phenotypic variability of HHH patients.

Expression and characterization of recombinant uroporphyrinogen III synthase mutations causing congenital erythropoietic porphyria.. *L.F. Cunha, D.F. Bishop, R.J. Desnick.* Human Genetics, Mount Sinai School of Medicine, New York, NY.

The markedly deficient (but not absent) activity of uroporphyrinogen III synthase (UROS) results in congenital erythropoietic porphyria (CEP), an autosomal recessive disorder with marked phenotypic heterogeneity. Previously, genotype/phenotype assessments were based on UROS activity in unpurified lysates from a bacterial expression system that generates low amounts of the recombinant enzyme. To characterize the mutations, a new fusion protein expression system was used to obtain high yields of soluble UROS allowing rapid purification to homogeneity by affinity and conventional chromatography. Wild-type and mutant human UROS were expressed in the pSUMO vector as a fusion protein with the 14 kDa Small Ubiquitin-related MOdifier peptide (SUMO). Characterization of the mutations expressed in this system demonstrated the reduced specific activity of purified UROS and/or reduced yield of the mutant proteins. For example, purified UROS with the C73R mutation (one of the most severe phenotypes) had significant residual activity, but yielded less than 2% of wild-type protein. Likely protein miss-folding and/or protease instability additionally contributes to the residual activities in vivo. This rapid high-efficiency purification system will facilitate genotype / phenotype studies and guide efforts such as utilization of small molecule chaperones in studies of potential therapies.

A point mutation in metaxin 1 is associated with the N370S mutation in Gaucher disease. *M.E. LaMarca, N. Tayebi, E. Sidransky.* Clinical Neurogenetics, NIMH/NHGRI, NIH, Bethesda, MD.

The region of chromosome 1q21 encompassing the human glucocerebrosidase gene (GBA) is particularly gene rich, with 7 genes and 2 pseudogenes located within 85 kb. Metaxin 1 (MTX1), a novel gene believed to encode a part of the preprotein import complex of the mitochondrial membrane, is a convergently transcribed gene contiguous to the 3' end of the GBA pseudogene. A metaxin pseudogene is located in the 16 kb region between GBA and its pseudogene. To investigate whether alterations in the MTX1 gene could be associated with atypical presentations of Gaucher disease, the MTX1 gene was sequenced in patients with Gaucher and parkinsonian manifestations. A T>C point mutation in exon 7 of MTX1 leading to the amino acid change F202L was detected in 6 of 8 patients. It was noted that the MTX1 F202L mutation occurred only in those patients with an N370S allele in GBA, and was homozygous in the 2 patients who were homozygous for N370S. DNA from normal controls and from additional patients with Gaucher disease was screened to determine the frequency of the MTX1 F202L mutation. Using PCR amplification and direct sequencing of exon 7 of MTX1, 152 alleles from control individuals (72 Ashkenazi and 80 non-Ashkenazi) were screened for the MTX1 F202L mutation. Seven alleles (4.6%) were identified with MTX1 F202L, but none of the 7 had the GBA N370S. However, 16 GBA N370S homozygotes were all found to be MTX1 F202L homozygotes. Moreover, 27 non-Jewish patients with type 1 Gaucher disease who were N370S heterozygotes were also found to carry one allele with the MTX1 F202L mutation. Eight patients with type 3 Gaucher disease (without N370S) all lacked the MTX1 F202L mutation. The functional consequences of the MTX1 F202L alteration and its possible relationship to Gaucher disease remain to be elucidated. While the F202L in metaxin 1 may be a polymorphism, it does appear to be closely associated with the N370S mutation in glucocerebrosidase, and may be useful in tracing the evolution of the N370S allele.

Mutations in the 3' region of *DHCR7* and failure to normalize plasma cholesterol correlate with need for tube feeding in Smith-Lemli-Opitz syndrome. *L.S. Merkens*¹, *W.E. Connor*², *K.P. Battaile*¹, *C.L. Maslen*², *L.M. Linck*¹, *D. Lin*², *D. Flavell*², *R.D. Steiner*^{1,3}. 1) Pediatrics; 2) Medicine; 3) Mole&Med Genetics, Oregon Health & Sci Univ, Portland, OR.

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive condition caused by mutations in the gene encoding the final enzyme in cholesterol synthesis, 7-dehydrocholesterol D⁷reductase (*DHCR7*). Low cholesterol and high 7-dehydrocholesterol (7DHC) concentrations contribute to the pathogenesis. We studied whether dietary cholesterol (egg yolk) supplementation would increase cholesterol and decrease 7DHC plasma concentrations. 16 subjects with SLOS were enrolled. Feeding difficulties are common in both human SLOS and in the SLOS mouse models. 7 of the 16 subjects in the study were unable to take adequate caloric intake orally and required tube feeding. We identified mutations in *DHCR7* in 15 of the subjects and measured plasma sterols at baseline (after ≥ 3 wk of an essentially cholesterol-free diet) and after ≥ 6 months of cholesterol supplementation. Plasma cholesterol increased significantly in response to supplementation. The 9 subjects who were orally fed had significantly higher plasma cholesterol at baseline and after supplementation (83 and 126mg/dl, means) vs. subjects who were tube fed (50 and 74mg/dl) $P < .001$. After supplementation 8 of the orally fed subjects had cholesterol concentrations above 100mg/dl. Only 1 of the tube fed subjects had plasma cholesterol that was above this limit. 7DHC concentrations were significantly lower at baseline and after supplementation in orally fed subjects (6.9 and 5.2mg/dl) vs. tube fed subjects (11.8 and 12.1mg/dl) $P < .002$. Even 5mg/dl, is 500 times greater than normal (0.01mg/dl). None of the orally fed subjects, but 5 of the 7 tube fed subjects had at least one missense mutation in the 3' region of *DHCR7*, which altered an amino acid within the last 65 amino acids. Mutations in the 3' region of *DHCR7* may predispose to feeding difficulties. Therapies in addition to cholesterol supplementation may be necessary to normalize the concentration of plasma (and tissue) sterols, particularly in tube fed patients.

Comparison of Baseline Clinical Characteristics Between Patients (pts) with Type I Gaucher Disease (GD) with Multi-point Genotype Mutations and Pts with More Common Genotype Mutations: A Report from the Gaucher Registry. *P. Kaplan, H. Andersson, J. Charrow, E. Kolodny, P. Mistry, G. Pastores, B. Rosenbloom, C.R. Scott, R. Wappner, N. Weinreb, A. Prakash-Cheng.* ICGG, Cambridge, MA.

Multi-point mutations [mpm] (L444P+A456P+V460V; D409H+L444P+A456P+V460V) in the glucocerebrosidase gene are generally associated with more severe Type I GD. This GR report compares baseline clinical characteristics of Type I GD pts with a mpm to pts with the 3 commonest genotypes. All Type I pts with reported genotypes and baseline clinical characteristics were summarized. Baseline is the value closest to 1st infusion for pts receiving Enzyme Replacement Therapy [ERT] or most recent value for pts Not on ERT. Among 1518 pts with genotype data in January 2002, the commonest genotypes were N370S/N370S (34%), N370S/L444P (17%), and N370S/84GG (11%). 2% of 1518 patients reported a mpm. Pts with unknown/rare alleles were not included in this summary. The proportions of pts in each genotype group with total splenectomy at baseline were 13% in N370S/N370S, 21% in N370S/L444P, 31% in N370S/84GG, and 21% in multi-point genotype mutations. Mean baseline hemoglobin (g/dL) was 12.2 in N370S/N370S, 11.7 in N370S/L444P, 11.6 in N370S/84GG, and 11.2 in multi-point genotype mutation. Mean baseline platelets ($\times 10^3/\text{mm}^3$) was 124 in N370S/N370S, 125 in N370S/L444P, 154 in N370S/84GG, and 129 in multi-point genotype mutation. Mean baseline liver volume [multiples of normal (MN)] was 1.4 in N370S/N370S, 1.7 in N370S/L444P, 2.2 in N370S/84GG, and 1.8 in multi-point genotype mutation. Mean baseline spleen volume (MN) was 10.4 in N370S/N370S, 16.3 in N370S/L444P, 20.1 in N370S/84GG, and 15.9 in multi-point genotype mutation.

Conclusion: The baseline clinical characteristics of Type I pts with a multi-point genotype mutation are comparable to Type I pts with the N370S/N370S, N370S/L444P, and N370S/84GG mutations. Although mean baseline clinical values among these 4 groups of pts are comparable, variation among individual results suggests that genotype cannot be used as the single indicator of disease severity and that the relationship between genotype and phenotype in GD is not fully understood.

Clinical findings in MCAD patients heterozygous for the common mutation identified by MS/MS newborn screening. *S.E. McCandless¹, D. Millington², B.S. Andresen³, N. Gregersen³, J. Muenzer¹, D.M. Frazier¹.* 1) Depts of Pediatrics & Genetics, Univ North Carolina, Chapel Hill, NC; 2) Dept of Pediatrics, Duke Univ, Durham, NC; 3) Faculty of Health Sciences, Århus Univ, Århus, Denmark.

Through May 2002, North Carolina has identified 41 individuals with medium chain acyl-CoA dehydrogenase (MCAD) deficiency from 557,720 MS/MS newborn screening (NBS) samples. Accumulation of diagnostic acylcarnitines (AC) in plasma or acylglycines in urine confirmed affected status. Of the 41, 30 (71%) are homozygous for the common G985A mutation. Eleven individuals have one or no copies of the common mutation. One African-American male has no recognizable mutation found after sequencing the coding portion of the gene. He had classic biochemical findings in the newborn period (plasma AC8 and AC8/AC10 ratio both markedly elevated and diagnostic acylglycines in the urine). Ten presumed compound heterozygotes were found to have at least one copy of the common mutation. The second mutation has been identified in seven; three individuals are still being studied. All 10 were confirmed by plasma acylcarnitine profile collected at 2 to 25 days of age. Urine organic acids at various times were diagnostic in 6, and suggestive in 3, of 9 tested. No compound heterozygote had low plasma free carnitine concentrations prior to treatment with supplemental carnitine compared to 3/23 among the homozygotes tested. Significant differences in initial metabolic measures between compound heterozygotes and homozygotes include (95% C.I.): NBS AC8 to AC10 ratio 6.0 (3.6-8.4) vs. 10.2 (9.2-11.2), plasma AC8 1.86 (0.83-2.89) mmol/L vs. 3.91 (3.24-4.59), and plasma AC8 to AC10 ratio 5.6 (3.3-7.9) vs. 11.6 (9.9-13.4). No compound heterozygote had neonatal hypoglycemia (compared to 1/30 of the homozygotes). There have been no episodes of documented hypoglycemia in the compound heterozygotes, but several admissions for persistent vomiting. Clinical outcomes have been excellent for both groups to date, with no deaths or individuals with developmental delay. In spite of differences in metabolite profiles, we are not able to identify a significant difference in the clinical course between the two groups when on treatment.

A new approach for positional cloning using cDNA clones: The discovery of *HPS3* and *OPA3*. Y. Anikster^{1, 2}, M. Huizing², R. Kleta², P.D. Anderson², W.A. Gahl². 1) Metabolic Disease Unit, Pediatrics, Chaim Sheba Medical Center, Tel- Hashomer, Israel; 2) Section on Human Biochemical Genetics, NICHD, NIH, Bethesda, Maryland.

Linkage analysis for central Puerto Rican Hermansky-Pudlak syndrome (HPS) patients and for Iraqi-Jewish 3-methylglutaconic aciduria (MGA) patients identified the general locations for the genes causing these disorders. Traditionally, this localization would be followed by gDNA sequencing of candidate genes in the region. We have successfully used an alternative approach. PCR amplification was attempted for each candidate gene using cDNA from patient and control fibroblasts.

For HPS, three cDNA clones with unknown function served as candidate genes. For one of them, we were unable to amplify patient cDNA, but could amplify control cDNA. We next checked for the presence of an mRNA transcript on a northern blot and found none. We concluded, correctly, that this cDNA was the *HPS3* gene. For MGA, we identified four candidate genes. Two were not expressed in fibroblasts, and their genomic DNA was sequenced. The third and fourth were expressed in fibroblasts from controls, but one was not expressed in patients. This gene was identified as *OPA3*.

In *HPS3* the central Puerto Rican mutation was a 3904-bp deletion, and in *OPA3*, the mutation involved a splice site. Since approximately 30% of human gene mutations result in reduced mRNA expression, our approach can often be effective, and is more efficient than extensive sequencing.

In addition to its use for positional cloning, demonstrating absent expression is helpful in identifying genes mutated in certain syndromes. We used this method to rule out *MYO5A* mutations and rule in *RAB27A* mutations in a specific case of Griscelli syndrome.

3-Methylglutaconic aciduria type III in a non Iraqi-Jewish patient. A. Jeong¹, M. Lalor¹, P.D. Anderson¹, R. Kleta¹, F. Skovby², M. Huizing¹, W.A. Gahl¹, Y. Anikster^{1,3}. 1) Section on Human Biochemical Genetics, HDB, NICHD, NIH, Bethesda, MD; 2) Department of Clinical Genetics, Rigshospitalet, Copenhagen, Denmark; 3) Sheba Medical Center, Tel Hashomer, Israel.

Based on clinical and molecular findings, 3-methylglutaconic aciduria (MGA) has been divided into four different disease categories: type I (3-methylglutaconyl-CoA hydratase deficiency), type II (Barth syndrome), type III (Costeff Optic Atrophy syndrome), and type IV (unclassified). MGA type III is a neurophthalmologic syndrome consisting of early (infantile) bilateral optic atrophy, ataxia and mild spasticity. The urinary excretions of 3-methylglutaconic and 3-methylglutaric acids are elevated in type III MGA. The presence of the disorder in an Iraqi-Jewish genetic isolate led to mapping of the OPA3 gene to chromosome 19q13.2-q13.3, followed by isolation of the gene itself. OPA3 consists of two exons and codes for a peptide of 179 amino acids. Iraqi-Jewish patients with type III MGA are homozygous for a splice site founder mutation in OPA3 (IVS1-1G>C) that abolishes mRNA expression in fibroblasts. To date, MGA type III has been assigned only to patients of Iraqi-Jewish ancestry. Our patient is a 14-year old boy whose Kurdish-Turkish parents are first cousins. Dysmorphic features were never noted. The patient walked at 11 months but the gait was unsteady at 16 months. CT of the brain was normal. Organic acid analysis of the urine revealed elevated 3-methylglutaconic acid and 3-methylglutaric acid. Major motor milestones were reached in spite of non-progressive ataxia, mild hypotonia and hypermobility. A brain magnetic resonance image revealed atrophy of the optic nerves, in both their retrobulbar and intracranial pathways. There was no cardiomyopathy, and the peripheral blood smear was normal. In this patient, previously assigned to the MGA type IV group, we found an in-frame 18 bp deletion in exon 2 of OPA3 (320-337del) leading to a shortened protein of 173 amino acids. We conclude that type III MGA also occurs in patients of non Iraqi-Jewish ancestry, and should be considered in patients with type IV MGA who have infantile optic atrophy and ataxia.

Mutations in the FTCD gene causing glutamate formiminotransferase deficiency. *D.S. Rosenblatt*^{1,2,3,4}, *J.F. Hilton*³, *K. Christensen*⁵, *T.J. Hudson*^{1,2,6}, *B.A. Raby*^{2,6}, *X. Estivill*⁷, *S. de la Luna*⁷, *R.E. MacKenzie*⁵. 1) Departments of Human Genetics; 2) Medicine; 3) Biology; 4) Pediatrics; 5) Biochemistry, McGill University, Montreal, Quebec, Canada; 6) Montreal Genome Centre; 7) Genomics Regulation Center, Barcelona, Spain.

Glutamate formiminotransferase deficiency (GFTD) (OMIM 229100) is an autosomal recessive inborn error of folate metabolism. A severe phenotype includes high levels of formiminoglutamate (FIGLU) excretion, megaloblastic anemia and developmental delay. A milder phenotype is characterized by even higher levels of FIGLU excretion but only mild developmental delay. Human formiminotransferase-cyclodeaminase consists of a tetramer of dimers, with dimerization essential for both formiminotransferase (FT) and cyclodeaminase (CD) activity. The human FTCD gene consists of 15 exons and is located on 21q22.3. Genomic DNA was isolated from 3 patients with suspected GFTD and each of the FTCD exons was amplified by PCR followed by direct sequencing. Cell lines from two siblings were heterozygous for mutations c457C®T and c940G®C. The c457C®T mutation changes a conserved arginine to a cysteine (R135C) in a loop involved in the binding of tetrahydrofolate to the FT enzyme domain. The c940G®C mutation converts an arginine to a proline (R299P) in a helix essential for dimerization of the FT domain. The third patient was either homozygous or hemizygous for a c1033insG mutation. All three mutations were absent in 200 control alleles. The c457C®T (R135C) and c940G®C (R299P) mutations were introduced into an expression vector containing the porcine FT domain with a C-terminal six histidine tag using overlap PCR. The proteins were expressed in *E. coli* (BL21DE3) and purified using a NiNTA column. Assays of R135C and R299P showed 61% and 57% of wild type activity respectively. A western blot of the expressed crude extract of the c1033insG mutations showed that the protein was truncated, completely lacking the CD domain. Assay of the crude extract for FT activity showed that the truncated protein was active. These findings represent the first demonstration that mutations in the human FTCD gene underlie GFTD.

Characterization of the normal cDNA for the canine *N*-acetyl- α -D-glucosaminidase gene, the gene defective in mucopolysaccharidosis IIIB. *N.M. Ellinwood*¹, *P.S. Henthorn*², *U. Giger*², *M.E. Haskins*¹. 1) Dept Pathobiology, Univ Pennsylvania, Philadelphia, PA; 2) Dept Clinical Studies, Univ Pennsylvania, Philadelphia, PA.

Mucopolysaccharidosis (MPS) III is a severe neuropathic lysosomal storage disease involving a failure of normal heparan sulfate catabolism. A canine form of MPS IIIB has been identified in Schipperke dogs, and we report herein on the normal cDNA for the canine *N*-acetyl- α -D-glucosaminidase (NAGLU) gene.

Canine MPS IIIB, characterized by deficient NAGLU activity, results in severe neuropathic disease, with clinical signs of diffuse cerebellar disease becoming evident in affected dogs as young adults. As a first step in defining the genetic basis of this disease model we isolated the normal canine NAGLU cDNA. The sequence extended from position -7 from the first ATG (in the context of a strong initiator site from -3 to +4), to the polyadenylation signal. The open reading frame of 2244 nucleotides bears substantial identity to human (87%) and mouse (84%) NAGLU sequences. The predicted protein comprises 747 amino acids, again with considerable identity to the human (83%) and mouse (79%) proteins. The amino terminus contains a stretch of 23 hydrophobic amino acids consistent with a signal peptide. The predicted mature protein, minus any possible carbohydrate residues, comprises a protein of 724 amino acids with a mass of 81.2 kDa.

The identification of the normal canine NAGLU sequence will allow for the identification of the disease causing mutation, and hence development of DNA based diagnostic, to be used to manage a research colony, and to help identify carrier animals in the pedigreed Schipperke dog population. The normal cDNA will also prove useful in developing this model as a means to evaluate enzyme replacement and gene therapies. A breeding colony of 6 dogs has been produced and the first carrier-by-carrier matings are under way. This colony will allow for the assessment of the pathogenesis of and therapy for MPS IIIB. Supported by NIH Grants RR02512 and RR007063 and a grant from The National MPS Society.

Renal Involvement in Gaucher Disease: Pathogenesis and Natural History. *O. Goker-Alpan*¹, *N. Tayebi*¹, *P.S. Kishani*², *H. Rosenbaum*³, *D. Rubinger*⁴, *E. Sidransky*¹. 1) NIMH/NIH, Bethesda, MD; 2) Duke University, Durham, NC; 3) Rambam Medical Center, Haifa, Israel; 4) Hadassah University, Jerusalem, Israel.

Gaucher disease (GD) is characterized by multi-organ involvement due to the accumulation of glucocerebroside in the cells of the monocyte-macrophage system. While hematopoietic, skeletal and hepatic involvement is frequently encountered, renal disease has been considered rare. To determine the incidence and nature of kidney involvement and to further investigate the pathogenesis of kidney disease in GD, we examined clinical and molecular data of 135 patients and identified 8 patients with renal involvement. Overall, 6% of our patient population had kidney involvement. There was no single common genotype nor pathological diagnosis shared among the 8 patients. The kidney disease did not correlate with the severity of GD or the patients genotype. Proteinuria was the most common presenting symptom (7/8). In the majority it was in the range encountered in nephrotic syndrome (4/7). There were two major presentations: isolated proteinuria (3/8) and proteinuria associated with immune activation (3/8). The course of the patients with isolated proteinuria was benign. However the three patients with immune mediated renal disease developed renal failure and one died of end stage renal disease. Two biopsy specimens, one from a patient with isolated proteinuria and the other with end stage renal disease with decreased complement levels were analyzed by light microscopy, EM and immunofluorescent studies. The light microscopy and EM failed to detect any Gaucher cells in the first patient. However on immunofluorescent studies there was no staining for glucocerebrosidase (GC) in the glomeruli, suggesting GC deficiency might have altered glomerular permeability and cause proteinuria. In the second patient, Gaucher cells were detected in the glomeruli on EM. In this patient, the release of cytokines from macrophages primed with glucocerebroside might have triggered a cascade of immune responses, causing irreversible renal damage. These two cases illustrate that there may be different mechanisms involved in pathogenesis of kidney disease in GD.

Enzyme replacement therapy in Japanese Fabry disease patients. *Y. Eto¹, T. Ohashi¹, Y. Utsunomiya¹, M. Fujiwara¹, A. Mizuno², K. Inui³, N. Sakai³, T. Kitagawa⁴, Y. Suzuki⁵, S. Mochizuki¹, M. Kawakami¹, T. Hosoya¹, M. Owada⁴, H. Sakuraba⁶, H. Saito⁷.* 1) Tokyo Jikei Univ Sch Med; 2) Chubu Nat'l Hosp; 3) Osaka Univ Sch Med; 4) Nihon Univ; 5) Int'l Univ Health and Welfare; 6) Tokyo Metro Inst Med Sci; 7) Nat'l Childrens Hosp.

Fabry disease (Galactosidase A deficiency) is an X-linked hereditary disorder that leads to the pathologic accumulation of globotriaosylceramide (GL-3) in cellular lysosomes, particularly in the vascular endothelium of the kidney, heart, and brain. This results in premature death due to the failure of these organ systems. The results of a Phase 3 clinical trial with recombinant human α -galactosidase (r-haGAL, Fabrazyme®, Genzyme Corp) in Fabry disease patients showed safe and effective clearance of GL-3 from the capillary endothelial cells of the kidney, heart, and skin. Here we report the results of an open-label phase 2 study of Fabrazyme therapy in 13 male Japanese Fabry patients. Endpoints included GL-3 clearance from kidney and skin vasculature measured by light microscopy (LM) and ELISA, and Quality of Life improvement. Patients were 16-34 years of age (mean 26.6) and were treated bi-weekly with 1 mg/kg of Fabrazyme for 20 wks. At 20 wks, 12/13 (92%) patients achieved reduction of GL-3 accumulation in both kidney and skin capillary endothelial cells to normal or near-normal levels ($p < 0.001$). Median kidney and plasma GL-3 levels decreased by 51.9% ($p = 0.003$) and 100% ($p < 0.001$) respectively, as measured by ELISA; renal function remained normal based on serum creatine levels and creatinine clearance. Fabry pain and quality of life were measured with the Short Form McGill Pain and SF-36 Health Survey questionnaires, respectively and improvement over baseline was observed in multiple categories. Related adverse events were mostly infusion-associated (fever and rigors). As expected, IgG antibody formation was observed in 11/13 (85%) patients, but resulted in no effect on treatment response. No IgE antibody formation was detected. The results suggest that treatment with r-hGAL is safe and effective in Japanese patients with Fabry disease.

Effective enzyme replacement therapy of murine galactosialidosis using insect cell-expressed PPCA and neuraminidase. *E.J. Bonten, J.N. Toy, L. Mann, J. Knowles, H. Hu, A. d'Azzo.* Department of Genetics, St Jude Child Res Hosp, Memphis, TN.

Galactosialidosis (GS) belongs to the type of lysosomal storage disorders (LSDs) which affects primarily cells of the reticuloendothelial system. The disease is caused by a defect in protective protein/cathepsin A (PPCA) that results in the secondary deficiency of neuraminidase (NEU1) and b-galactosidase. Early onset patients present with both somatic manifestations and neurodegeneration while late infantile/juvenile patients have only a systemic disease. These patients, as many other non-neuropathic LSD patients, could benefit from enzyme replacement therapy (ERT) if the therapeutic protein is poised for receptor-mediated uptake by affected cells. Enzymes produced in insect cells naturally expose core-type mannose residues and are specifically internalized by macrophages and other cell types via the mannose receptor. These recombinant enzymes could, therefore, be used for ERT in lysosomal diseases with a primary involvement of macrophages. We used the mouse model of GS to test this hypothesis. Cultured GS-macrophages effectively internalized both insect cell-produced PPCA and Neu1, restoring their catalytic activities up to 60-fold the control values. Internalization occurred via the mannose receptor and was inhibited by mannan, a competitive substrate for this receptor. Short term treatment of young GS mice by intravenous administration of a mixture of both enzymes led to full restoration of catalytic activities in visceral organs, and almost complete resolution of lysosomal storage in kidney, liver, and spleen. The injected enzymes were detected by Western blotting or by immunohistochemistry in the liver, spleen, kidney, skeletal muscle, heart, adrenal gland, and choroid plexus. The results of these studies suggest that the use of a BV-produced recombinant enzyme alone or combined with other therapeutic means could afford amelioration of the systemic phenotype in non-neuropathic GS patients, and other LSDs affecting primarily the reticuloendothelial system. (Supported by NIH grants DK52025 and GM60950).

Patients Affected With Fabry Disease Have An Increased Incidence of Progressive Hearing Loss and Sudden Deafness: A Study of Twenty-Two Hemizygous Male Patients. *D.P. Germain¹, P. Avan², A. Chassaing¹, P. Bonfils².*
1) Dept Genetics, European Hospital Georges Pompidou, Paris, France; 2) Auditory Research Laboratory, European Hospital Georges Pompidou, Paris, France.

Objective: Fabry disease (FD, OMIM 301500) is an X-linked inborn error of glycosphingolipid metabolism due to the deficient activity of alpha-galactosidase A, a lysosomal enzyme. While the progressive systemic deposition of uncleaved glycosphingolipids throughout the body is known to have protean clinical manifestations, few data are available regarding the cochlear involvement. **Methods:** We non-invasively investigated cochlear functions in 22 consecutive hemizygous males (age 19-64 years, mean 39) affected with classic FD. Conventional audiometry, tympanometry, ABR audiometry, otoacoustic emissions were performed in all patients, together with medical history record and physical examination as part of an exhaustive baseline evaluation prior to enzyme replacement therapy. **Results:** A total of 12 patients (54.5%) with classic FD were found to have abnormal audition. Five patients had progressive hearing loss and seven patients (32%) experienced sudden deafness. In addition, a hearing loss on high-tone frequencies was found in 7 out the 10 remaining patients without clinically significant deafness, despite their young age at time of examination. The incidence of hearing loss appeared significantly increased in FD patients with kidney failure ($P < 0.01$) or cerebrovascular lesions ($P < 0.01$), whereas there was no correlation with left ventricular hypertrophy. In addition, tinnitus aurium was found in six patients (27%). **Conclusion:** This is the first evidence of a high incidence of both progressive hearing loss and sudden deafness in a cohort of male patients affected with classic Fabry disease. The exact pathophysiologic mechanism of the cochlear involvement deserves further studies.

Making the diagnosis of cystinosis. *W.A. Gahl, I. Bernardini, C.R. Hermos, R. Kleta.* Section on Human Biochemical Genetics, HDB, NICHD, NIH, Bethesda, MD.

Nephropathic cystinosis is an autosomal recessive disorder due to deficient transport of free cystine out of lysosomes. Cystine accumulation causes renal tubular Fanconi syndrome (FS) in infancy, glomerular failure by 10 years of age, and other systemic complications. Early diagnosis is critical, because an effective therapy is available. Oral cysteamine, given every 6 hours at doses of 1.3-1.95 g/m² of free base or 60-90 mg/kg per day, delays or prevents renal deterioration and enhances growth if begun in the first 2 years of life. Recent experience with NIH patients points to possible pitfalls in the diagnosis of cystinosis. First, cystinosis patients can have significant proteinuria, and this finding should not militate against the diagnosis. FS is widely recognized to consist of impaired proximal tubular functions, e. g., reabsorption of glucose, phosphate, bicarbonate, and amino acids, the latter providing the most sensitive parameter for detecting the FS. However, protein reabsorption is also compromised in FS. Normally, albumin is filtered but nearly completely reabsorbed by the proximal tubule. When cystinosis impairs reabsorption, a significant tubular proteinuria can occur. Glomerular and tubular proteinuria can be distinguished by urine protein electrophoresis, and this can prevent unnecessary kidney biopsies. Second, corneal cystine crystals may be absent from cystinosis patients prior to one year of age, but are present in all affected individuals after 16 months of age. This slit lamp finding can make the diagnosis if noted by an experienced ophthalmologist. Third, leucocyte cystine measurements provide the gold standard for diagnosing cystinosis. Normal values are less than 0.2 nmol half-cystine/mg protein, and cystinosis values are ~3-20. For cystine determinations, the cystine binding protein assay is superior to standard amino acid analysis. Because artifacts underestimate cystine concentrations, any values above 0.2 should raise suspicion of cystinosis. These artifacts include increasing the protein denominator by red cell contamination or isolating lymphocytes (which store only 3-5 fold normal cystine in cystinosis) instead of polymorphonuclear leucocytes (which store 50-100 fold normal).

Characterization of the Functional Disability in Patients with Late Onset Glycogen Storage Disease type II (GSD II) Using a Modified Version of the Pediatric Evaluation of Disability Inventory (PEDI). *S.M. Haley¹, M.A.*

Fragala¹, A. Skrinar², E. Ponce². 1) Sargent College of Health and Rehabilitation Sciences, Boston University, Boston, MA; 2) Genzyme Corporation, Cambridge, MA.

GSD II is caused by deficiency of the lysosomal enzyme acid alpha glucosidase. Late onset GSD II presents as a progressive disease of the trunk, limb girdle, and respiratory muscles. The goal of this study is to characterize the functional status of patients with late onset GSD II using a GSD-II specific functional outcome measure. **Methods:** The PEDI is a standardized instrument developed to assess the functional self-care and mobility skills of children 6 months to 7.5 years of age. For this study, the PEDI was revised to include less challenging head, trunk and upper extremity items for children with severe functional loss and more challenging gross motor items for children with milder symptoms. Telephone interviews were done by parent proxy on a convenience sample of 30 patients (mean age 7.7 5.6 years; range 0.4-22.1). The sample was predominantly male (76.7%) and Caucasian (86.7%) originating from the U.S. (24), Canada (2), Germany (1), Spain (1), England (1), and Denmark (1). One parent required a translator to respond to the English survey. **Results:** Two-thirds of the sample was non-ambulatory and an additional 10% was able to ambulate only with assistance of a device. 2 out of 30 patients were able to participate in age-expected sports/school activities. 3 out of 30 patients had severe motor loss and could accomplish only a few head control items in supine. One-half of the children used a ventilator during the day, and only 23% of the school-age children attended school. Although the average chronological age of the sample was 7.7 years, the actual average age performance was 2.4 years (range 0.5-4.6) for self-care skills and 1.4 years (range 0.4-7.5) for mobility skills. **Conclusion:** Patients with late onset GSD II presented with heterogeneous functional profiles, although most had severe functional self-care and motor losses. These findings provide a rationale for the modification of instruments to capture the wide range of functional skills observed in patients with late onset GSD II.

Cystinosis due to a CTNS deletion having breakpoints within Alu repeats. *R. Kleta, D.L. Fitzpatrick, M. Rausche, C. Lucero, C.R. Hermos, Y. Anikster, I. Bernardini, W.A. Gahl.* Section on Human Biochemical Genetics, HDB, NICHD, NIH, Bethesda, MD.

Cystinosis is an autosomal recessive disease with intralysosomal cystine accumulation leading to kidney failure within the first decade of life and several nonrenal complications if a renal transplant is performed. The gene CTNS, mutated in cystinosis, codes for the protein cystinosin, which contains 367 amino acids and 7 transmembrane domains. Cystinosin is the lysosomal cystine carrier defective in cystinosis. The disorder occurs in all ethnic groups and, to date, 56 different CTNS mutations have been described, including promoter, missense, nonsense, deletion, insertion, and splice site mutations. The genomic area covering the CTNS gene consists of ~25 kb and 12 exons. A 200,237-bp region encompassing CTNS consists of ~42% repetitive elements, of which 30 % are Alu-like repeats. Such repeats have been implicated as the cause of mutations, via homologous recombination, in hereditary disease. The most prevalent CTNS mutation is a 57,257 bp deletion removing exons 1 to 10, the promoter region and an adjacent gene of unknown function called CARKL. The breakpoints of this deletion, in exon 10 and ~45-kb upstream of the CTNS start codon, are unrelated to repetitive elements. We investigated a 20-year old Mexican patient with classical nephropathic cystinosis who was heterozygous for the 57-kb deletion. We could not obtain a PCR product using his genomic DNA and primers in exons 4 and 5, pointing to another large deletion in the patient's second allele. In fact, it was extremely difficult to obtain any PCR products within this highly repetitive region. By sequential PCR amplification between exons 3 and 6, we eventually defined both breakpoints within two separate Alu repeat regions of 290 bp and 279 bp. The breakpoints match exactly the beginning of a new motif within the two Alu repeats, which exhibits 87% homology. This previously undocumented deletion encompasses 6114 bp between intron 3 and intron 5 of CTNS. Our patient's mutation represents the third largest CTNS deletion known and the first Alu repeat-based deletion in CTNS.

Mutation analysis and X-inactivation studies in the α -galactosidase A gene. *A. Morrone*¹, *C. Cavicchi*¹, *T. Bardelli*¹, *D. Antuzzi*², *D. Rigante*², *R. Parini*³, *M. Di Rocco*⁴, *S. Feriozzi*⁵, *O. Gabrielli*⁶, *R. Barone*⁷, *M. Aricò*⁸, *R. Ricci*², *E. Zammarchi*¹. 1) Dept Pediatrics, Univ Florence; 2) Catholic Univ, Rome; 3) ICP Hosp, Milan; 4) Gaslini Inst, Genova; 5) Belcolle Hosp, Viterbo; 6) Ped Clinic, Ancona; 7) Dept Ped, Catania; 8) Dept Dermatology, Palermo, Italy.

Fabry disease is an X-linked lysosomal storage disorder due to a deficiency of the enzyme α -galactosidase A (GLA). It is a multisystemic disease characterized by renal failure and angiokeratoma. Heterozygous females can be asymptomatic or clinically affected. Up to now more than 200 mutations have been identified in the GLA gene, mapped on Xq22. We report the molecular studies of 14 Italian Fabry male patients affected by the classic form of the disease and 7 carriers from 11 unrelated families. Four new (L167P, c617-618delTT, c126-127insCATG, c946delG) and seven known (P40L, R227Q, W236C, C378Y, R220X, Y356X, IVS3+1G>A) mutations were identified in the patients' GLA gene. An aberrant GLA transcript c486-547del62bp, that leads to an early stop codon, was detected in a male patient carrying the IVS3+1G>A splicing defect. These results confirm the molecular heterogeneity also in the Italian Fabry patients. We also report X-inactivation studies on 7 carriers with a positive family history for Fabry disease in males. Four females showed a random X-inactivation pattern and three were skewed. One of the skewed females was a manifesting carrier with angiokeratoma. The other two skewed carriers were two sisters and only one was a manifesting carrier. In these sisters the skewed X-inactivation pattern in favour of the mutant allele was detected in the manifesting carrier, while a skewed pattern in favour of the wild type allele was detected in the other. In these carriers the X-inactivation studies suggest a correlation between clinical manifestations and the skewing of X-inactivation. It is important to emphasize the characterization of Fabry patients genotype with the aim of a genotype/phenotype correlation and the carrier status assignment. We would like to point out the importance of X-inactivation studies that could be helpful in predicting the carriers' phenotype and give useful indications for therapeutic management.

Genes required for cholesterol biosynthesis and lysosomal function are upregulated over time in response to sucrose-induced vacuolation in normal fibroblasts. A. Helip Wooley, J.G. Thoene. Human Genetics, Tulane Univ Sch Medicine, New Orleans, LA.

Normal human fibroblasts cultured in the presence of 100 mM sucrose form lysosome-derived vacuoles via pinocytic uptake and storage of sucrose, due to the absence of invertase in these cells. The protein synthesis inhibitor cycloheximide significantly decreased apparent vacuolation, observed by phase microscopy and ^{14}C -sucrose uptake and storage, in a time and concentration dependent manner. 50 mM cycloheximide decreased ^{14}C -sucrose uptake by 36%; at 48 h, $P < 0.004$. Changes in gene expression following 24 hours of sucrose induced-vacuolation were examined in triplicate using the Affymetrix HGU95A microarray. Thirty-seven genes were increased an average of 2-fold or greater, relative to matched controls including cholesterol biosynthesis and metabolism genes (mevalonate pyrophosphate decarboxylase (MPD) (16.8 fold \pm 13), delta7-sterol reductase (2.7 \pm 0.8), LDL receptor (3.0 \pm 0.3) and 3-HMG-CoA reductase (2.0 \pm 0.1)), lysosome related genes (neuraminidase (2.4 \pm 0.4), CLN3 (gene for Juvenile Batters disease) (2.2 \pm 0.8) and CLC-7 a lysosomal chloride channel (2.0 \pm 0.5)), and a protein (Rab7L1) with 30% homology to the small GTP-binding protein Rab7, implicated in late endosome and lysosome fusion (2.1 \pm 0.4). Gene expression was also examined by microarray following 12, 24, 72 or 120 hours of sucrose exposure and compared to both time 0 control and matched time controls. The majority of increased genes, including those mentioned above, demonstrated little or no change at 12 hours but were increased by 24 hours and at all timepoints thereafter. These changes were generally greater when compared to the T_0 control but were similar to time matched control results. This is illustrated by MPD which increased 0, 13, 16 and 17 fold at 12, 24, 72 and 120 hours versus T_0 and 1.4, 13, 13, and 4 fold versus time matched controls. These findings suggest that cellular mechanisms exist to detect perturbations in lysosomal physiology and respond by upregulating pathways necessary to accommodate these changes.

Experience with Safety Surveillance Programs for Diseases Requiring Enzyme Replacement Therapy. *M.J. Motta, R.D. Cintron.* Medical Affairs, Genzyme Corporation, Cambridge, MA.

Lysosomal storage disorders are caused by deficiency of catabolic enzymes leading to the accumulation of substrates within the lysosomes and causing function impairment. With the development of enzyme replacement therapy (ERT) an established safety surveillance program is essential to provide careful and timely medical review of reported adverse events (AEs). A proactive method to monitor antibody formation is also critical. **Methods:** Approximately 4,000 patients worldwide currently receive ERT for Gaucher, Fabry, MPS-I and GSD-II. AEs information is collected in the United States following FDA guidelines detailed in 21CFR 312.32 (clinical trials) and 21CFR 314.80 (post-marketing). Clinically significant changes observed in ophthalmic tests, echocardiograms, EKGs and routine blood chemistries among others are recorded and analysed. Patient sera are regularly monitored for antibody formation and to study immunologic tolerance over time. **Results:** Observed AEs generally fall within three main groups: infusion site reactions, immune-mediated reactions, and events secondary to the nature of the disease. Infusion site reactions are primarily local swelling/irritation and are often due to extravasation. Immune-mediated reactions often correlate with the development of antibodies to the exogenous protein. In this group of patients, infusion-associated reactions are possible, often predictable, found to be a function of protein load, and influenced by the rate of infusion. AEs secondary to the nature of the disease vary within the different LSDs. Factors affecting the nature and frequency of the latter include type, extent, and severity of the organ(s) involvement. **Conclusions:** Safety surveillance provides vital information on ERT and continuous information on the nature of the disease being treated. Infusion-associated reactions can be managed with conservative measures. The development of antibodies to exogenous proteins is anticipated in patients with little or no endogenous enzyme activity and does not preclude the safe continuation of ERT. In protein-based recombinant products, the extent and overall effect of immunogenicity remains an open question.

Neurologic complications of female and male patients with Fabry disease. *R.J. Hopkin^{1,3}, F.J. Samaha^{2,3}, H. Zhao¹, L. Bailey^{1,3}, G.A. Grabowski^{1,3}.* 1) Dept Human Genetics, Cincinnati Children's Hosp, Cincinnati, OH; 2) Dept Neurology, Cincinnati Children's Hosp; 3) Lysosomal Disease Center, Cincinnati Children's Hosp.

Fabry disease is an X-linked lysosomal storage disease caused by a deficiency in agalactosidase A. Twenty-three hetero- or hemi-zygotes (ages 20-61) were evaluated for CNS involvement using history, clinical examination, and CNS MRI. The most common abnormalities reported are summarized in the table. The vertigo and head aches were quite severe leading to hospitalizations and/or job loss for several participants. Hyperacusis was more common in heterozygotes than hemizygotes. Psychiatric diagnoses included schizophrenia (1 female, 2 male), antisocial behaviors (3 female, 3 male), and depression (4 female, 4 male). Although neurologic complications have previously been reported in Fabry disease, these findings are surprising since the age at onset was earlier than previously reported (teens for several participants). The frequency of complications was extremely high (90 percent had at least one clinically significant CNS complication). The equal frequency and severity of neurologic complications in hetero- and hemi-zygotes is surprising and significant. These findings have important implications in planning for and monitoring treatment needs of individuals with Fabry disease. The availability of enzyme therapy for Fabry disease provides added impetus to further delineate the spectrum of this disease especially in heterozygotes.

	MRI	Vertigo	Headache	Hearing	psych	neuro exam	stroke
Female	7/7	7/9	9/9	8/9	4/9	4/8	2/9
Male	9/12	11/14	9/14	4/13	6/14	5/13	3/14
Total	16/19	18/23	18/23	12/22	10/23	9/21	5/23

Report of the Fabry International Research Exchange (FIRE) Registry. *R.D. Steiner¹, C. Feist¹, T. Clemons² for the FIRE Registry Group.* 1) Oregon Hlth Sci U, Portland, OR; 2) EMMES, Rockville, MD.

Fabry disease is an x-linked glycosphingolipid storage disorder caused by deficient activity of the lysosomal enzyme α -galactosidase A. Globotriaoslyceramide (Gb3), the substrate for this enzyme, progressively accumulates in cells and tissues of affected patients causing pathology. The FIRE Registry collates data on the natural history of Fabry disease and response to therapy. As of 6/02, 68 patients (39 males (m) and 29 females (f)) have been enrolled at 17 centers across the US and overseas. The mean age of m = 32 ± 13 years(y), and f = 39 ± 17 y. Most (85%) were Caucasian. One m had received enzyme replacement therapy. The majority (54%) of m were diagnosed by mutation analysis, while many of the f (59%) were diagnosed by having an affected m first degree relative with the diagnosis confirmed by genotyping. The mean age of onset of Fabry symptoms was 9 ± 6 y in m and 16 ± 16 y in f. The mean age of diagnosis was 18 ± 12 y in m and 27 ± 17 y in f. The prevalence in m and f of the following signs and symptoms were: tinnitus (m-33%, f-30%); vertigo (m-22%, f-22%); hearing loss (m-28%, f-22%); angiokeratoma (m-72%, f-37%); corneal pathology (m-61%, f-41%); and pain (m-67%, f-52%). The prevalence of organ involvement was: stroke (m-11%, f-4%); proteinuria (m-28%, f-41%); renal insufficiency (m-22%, f-7%); HTN (m-17%, f-11%) and cardiomyopathy (m-6%, f-11%). Analysis of the occurrence of signs/symptoms by age and gender revealed that the earliest signs/symptoms in m were angiokeratomas, corneal pathology and neuropathic pain, while in f, corneal pathology and pain were the most common presenting signs/symptoms with angiokeratomas not reported in f until >25y. Proteinuria was common in m and f >25y, while renal insufficiency and cardiomyopathy were not reported until >35y in m and >44y in f. F with Fabry disease appear to have many if not all of the signs/symptoms seen in m, but typically present and are diagnosed almost 10y later than m. Organ involvement manifests approximately 10y later in f as well. In contrast to previous characterizations of Fabry as X-linked recessive, most, if not all women with a Fabry gene mutation exhibit signs or symptoms of disease.

A biochemical and histological mouse model for aggressive Gaucher disease. *B.M. Quinn¹, Y. Sun¹, D.P. Witte², G.A. Grabowski¹*. 1) Human Genetics; 2) Pediatric Pathology, Children's Hosp Medical Ctr , Cincinnati, OH.

Gaucher disease, an autosomal recessive trait, results from deficiency of the activity of the lysosomal enzyme acid b-glucosidase (BGC). This leads to the accumulation of glucosylceramide within the lysosomes of Gaucher storage cells contained in several organs, mainly the spleen and liver. Mice homozygous for V394L mutation of BGC showed < 20% of WT activity *in vitro* but no phenotype out to > 13 months. In humans Gaucher like disease also results from mutations in saposin C, an activator of BGC. Mice with a homozygous knock out of the saposin C precursor, prosaposin, die of neurological complications within one month. These mice can be rescued with a prosaposin transgene (PS-TG) driven by the low level general promoter for 3-phosphoglycerate kinase. This rescue leads to fertile mice with greatly delayed neurological complications and lifespans > 7 months. The visceral organs accumulate glycosphingolipids but appear histologically normal though out their lives. The combination of prosaposin knockout, the PS-TG and V394L homozygosity results in mice with considerable numbers of Gaucher storage cells in the spleen, liver and lungs by two months of age. Electron microscopy shows these cells to contain the characteristic braided structure of stored glucosylceramide. Lipid analysis by TLC confirmed accumulation of glucosylceramide in these tissues. These mice also manifest CNS clinical abnormalities. This represents a viable mouse model of aggressive visceral Gaucher disease associated with CNS deterioration and should be a useful therapeutic model.

Activation mechanism of saposin B. *M. Wang, X. Qi.* Dept Human Genetics, Children's Hosp Medical Ctr, Cincinnati, OH.

Saposin B is an activator for enzymatic hydrolysis of sulphatide by arylsulfatase A in lysosomes. Deficiencies of saposin B have been shown to result in metachromatic leucodystrophy. Some investigations have shown that unlike saposin C, saposin B activates the enzyme by interacting with substrate in lipid bilayers. In order to study the activation mechanism of saposin B, an in vitro detergent free activation assay and a fluorescent resonance energy transfer (FRET) system were exploited to measure the activation function of saposin B and interaction mechanism between saposin B and cerebroside sulfate. To clarify that the interaction between saposin B and cerebroside sulfate is related to the activation function of saposin B, three mutants (Y50A, Y54A, I8R) with different secondary structure as determined by circular dichroism spectroscopy were generated and produced. The circular dichroism spectra of mutant Y50A is same as the wild-type saposin B. According to the product amount in the in vitro assay analyzed on TLC, the activation of the Y50A mutant was the same as the wild type saposin B. The activation of the Y54A mutant was half of the wild type saposin B. The I8R mutant lost nearly all of its activation function. The rearranging tendency of cerebroside sulfate on the lipid bilayer was detected by the degree of the decreased intensity of fluorescent resonance energy transfer. The fluorescent intensity of the energy transfer between Dansyl-PS (1,2-Dioleoyl-sn-Glycero-3-Phospho-L-Serine-N-(5-dimethylamino-1-naphthalenesulfonyl)(Dansyl)) and LRh-CS (Lissamin-Rhodamin labeled Cerebroside Sulfate) was the wide-type Y50A < Y54A < I8R. The I8R showed no decrease in the intensity of fluorescent resonance energy transfer. The above results indicated that the FRET changes affected by saposin Bs were correlated with the activation function of saposin Bs. This suggested that the functionality of saposin B could be accomplishing by rearranging cerebroside sulfate on the lipid bilayer.

ER Stress-Mediated Apoptosis in the CNS of the GM1 Gangliosidosis Murine Model. *A. Tessitore, M. Del Pilar Martin, A. d'Azzo.* Genetics, St. Jude Child Res Hosp, Memphis, TN.

GM1 gangliosidosis is a lysosomal glycolipid storage disease caused by deficiency of the acid beta-galactosidase. The early infantile form of the disease is characterized by generalized central nervous system involvement, and is fatal in infancy. The mouse model of GM1 gangliosidosis develops features, which resemble the severe human condition, resulting in rapid progressive neurodegenerative symptoms, associated with neuronal cell death. Recent findings have suggested that activation of an endoplasmic reticulum (ER) stress response may be involved in the pathogenesis of neuronal cell injury in acute and chronic diseases of the brain. Conditions that alter the ER environment induce highly conserved cell stress responses, which include the transcriptional induction, translational attenuation, and degradation of unfolded proteins. In contrast, excessive and/or prolonged ER stress results in apoptosis. We found that activation of an ER stress-induced apoptotic pathway may contribute to the pathology of GM1 gangliosidosis. Increased mRNA levels of the stress-inducible transcription factor CHOP (C/EBP homologous protein) were detected in spinal cord samples from 5- to 8-month old GM1 gangliosidosis mice. In addition, mRNA levels of the ER resident protein GRP78 (78kD glucose-regulated protein) were inversely regulated during the course of the disease compared to CHOP. We also demonstrated transcriptional induction of caspase-12, a caspase family member predominantly localized in the ER and shown to be exclusively activated in ER stress-mediated cell death. The mRNA results were confirmed by immunoprecipitation and Western blot analysis of the caspase-12 protein. Together, the coordinate induction of CHOP and caspase-12 and the presence of apoptotic cells in spinal cord samples point to the activation of ER-stress apoptotic pathway in the CNS of GM1-gangliosidosis mice that might contribute to neurodegeneration. However, the mechanisms by which beta-galactosidase deficiency followed by massive lysosomal accumulation of GM1-ganglioside activate or render cells susceptible to ER stress cell death remain to be elucidated. (Supported by NIH grant DK52025).

Predictors of verbal dyspraxia in galactosemia. *L.J. Elsas II, A.L. Webb, R.H. Singh, M.J. Kennedy.* Division of Medical Genetics, Emory Univ School of Medicine, Atlanta, GA.

Classical galactosemia is an autosomal recessive disorder resulting from deficient galactose-1-phosphate-uridyl transferase (GALT). Verbal dyspraxia is one unexpected outcome of treated children with galactosemia. In this study, we validated a 2-hour breath test and evaluated four additional potential biochemical risk factors for verbal dyspraxia: mean RBC galactose-1-phosphate, highest RBC galactose-1-phosphate, mean urinary galactitol, and erythrocyte GALT activity, for their predictive value among a galactosemic population.

Thirteen controls and 42 patients with galactosemia took an oral bolus of ^{13}C -galactose and their breath was collected at 0, 90, 100, 110, and 120 minutes. The rate and cumulative percent dose (CUMPCD) of $^{13}\text{CO}_2$ in expired air was determined by dual isotope mass spectrometry at each time point. Twenty-eight of the 42 patients with galactosemia had <5% CUMPCD ("severe") and 14 had >5% CUMPCD ("variant"). Mutant hGALT alleles in the "severe" category were Q188R, K285N, D5kb, Y209C, V151A, and in the "variant" group were S135L, L189R, T138M, and D113N. A subset of 24 patients underwent formal speech evaluation; 15 (63%) had verbal dyspraxia. Those with dyspraxia had significantly lower CUMPCD values ($2.84 \pm 5.76\%$ vs $11.51 \pm 7.67\%$; $p < 0.008$), significantly higher mean erythrocyte galactose-1-phosphate (3.38 ± 0.922 mg/dL vs. 1.92 ± 1.28 mg/dL; $p = 0.019$) and mean urinary galactitol levels (192.4 ± 75.8 mmol/mol creatinine vs. 122.0 ± 56.4 ; $p = 0.048$) than those with normal speech. CUMPCD values below 5%, mean erythrocyte galactose-1-phosphate levels above 2.7 mg/dl and mean urinary galactitol levels above 135 mmol/mol creatinine were strongly associated with dyspraxic outcome with odds ratios of 21, 13, and 5, respectively.

We conclude that measurement of total body oxidation of galactose to CO_2 in a breath test is the most significant, sensitive, and specific predictor for dyspraxia among the abnormal biochemical parameters found in patients with galactosemia.

Remarkable recovery in adult-onset leigh syndrome with partial cytochrome *c* oxidase deficiency. P.C.

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Leigh syndrome (LS) is a heterogeneous disorder, usually due to a defect in energy metabolism. Symptoms typically commence in infancy or childhood. Progressive deterioration is the norm. We describe a 22-year old woman with acute presentation of LS and partial cytochrome *c* oxidase (COX) deficiency with dramatic clinical and neuroradiologic improvement following an acute encephalopathic episode.

The patient developed progressive spastic paraparesis one week following a febrile gastrointestinal illness. Over the next month, she developed complete aphonia, near quadriplegia, retinopathy and sensorimotor axonal polyneuropathy. Blood and CSF lactates were elevated. Vitamin and coenzyme Q therapy was instituted. MRI was normal at the onset of illness but later showed neurodegeneration in the basal ganglia. MRS showed increased lactate doublet and reduced N-acetylaspartate (NAA) in these areas.

Over the next five months the patient recovered muscle strength, sensation, the ability to ambulate with a cane, and spoke with only subtle dysarthria. Retinopathy continued. MRI showed decreased high signal intensity, with decreased lactate doublet and increased NAA on MRS.

COX activity was 48% and 58% of control mean in muscle mitochondria ($p < .05$) and skin fibroblasts respectively. All other respiratory chain activities and PDH were normal, ruling out other major causes of LS. Surf1 levels were normal. Sequencing of the three mtDNA-encoded COX subunits revealed no pathogenic mutations.

This is the first report of significant recovery in a patient with COX deficiency who met accepted criteria for LS. Her remarkable clinical improvement correlated with improvement of MRI and MRS.

The new mutation G105E in the OTC gene causes an acute onset in a man affected by ornithine transcarbamylase deficiency. *S. Bisanzi¹, A. Morrone¹, N. Gentiloni Silveri², M. Di Lillo², R. Ricci², D. Antuzzi², E. Zammarchi¹.* 1) Department of Pediatrics, University of Florence; 2) Catholic University, Rome, Italy.

Ornithine transcarbamylase deficiency (OTCD) is the most frequent urea cycle disorder, inherited as an X-linked trait. The OTCD phenotype is heterogeneous, ranging from neonatal onset hyperammonemic coma to asymptomatic adult. Here we report a 21 year old man with acute onset OTCD. He started complaining about abdominal pain and vomiting after a meal of fresh salmon and pasta, and he appeared a little disoriented. In the following few hours his symptoms were getting worse and he began to feel drowsy. Laboratory finding showed hyperammonemia and increased transaminases. Three days afterwards coma occurred. OTCD was suspected and sodium benzoate and bioarginine therapy was initiated. The patients ammonia blood level normalised but after 24 hours he went into a deep coma and he died after ten days. Direct DNA sequencing of the patient's OTC gene led to the identification of the transition c.314G>A that leads to the new amino acid change G105E. This mutation occurred in an evolutionary invariant residue, located in the polar domain of the OTC protein, and is likely to be important in the assembly of the trimer. It can be hypothesised that the G105E mutation could interfere with forming the active site. The late onset in this patient and the finding that his maternal grandfather is a hemizygous healthy carrier suggest that this mutation causes a partially functional protein (residual enzymatic activity 10%), although occurring in a conserved amino acid. It can be assumed that environmental factors provoke significant catabolic events able to upset the patients homeostasis and destabilize the protein complex interfering with residual OTC activity. In the patients mother the assay for X-inactivation showed a skewed pattern in favour of the mutant paternal X-chromosome. This pattern seems to contradict her asymptomatic carrier status and the negative allopurinol loading test, but it supports the hypothesis that phenotypic presentation can be caused by a combination of genetic and environmental factors.

Hermansky-Pudlak syndrome type-4 (HPS-4): Clinical and molecular characteristics. *P.D. Anderson, M. Huizing, M. Lynch, W.A. Gahl.* Heritable Disorders Branch, NICHD, NIH, Bethesda, Maryland.

Hermansky-Pudlak syndrome (HPS) is a rare disorder of vesicle formation characterized by oculocutaneous albinism, a bleeding diathesis due to storage-pool-deficient platelets, and in some patients lysosomal accumulation of ceroid lipofuscin. The disorder exhibits considerable locus heterogeneity; four genes in humans are now associated with HPS, and there are at least 14 mouse models with an HPS phenotype of reduced coat color, storage-pool-deficient platelets, and variable lysosomal dysfunction. The human homologue of the *light ear* mouse locus was recently identified as the fourth HPS-causing gene. We performed an extensive study on 23 unassigned HPS patients (not having HPS-1, HPS-2, or HPS-3 disease) to further characterize HPS-4 disease on clinical and molecular bases.

We identified the genomic organization of *HPS4*, including intron/exon boundaries, and found that the *HPS4* gene, located on chromosome 22q11.2-q12.2, produces at least two alternatively spliced mRNA transcripts which differ at their 5' ends. Using SSCP, we determined that 8 of our 23 at-risk patients had HPS-4. In the process, we identified 5 different *HPS4* mutations, including 1 frameshift and 4 nonsense mutations. Three alleles in 2 patients contained the previously reported Q698insAAGCA frameshift, and 1 patient was homozygous for the previously reported Q631X nonsense mutation. Four alleles in 3 patients contained the novel mutation R217X, and 2 siblings were compound heterozygotes for the novel mutations E138X and E222X. We also characterized the clinical phenotype of HPS-4 disease, demonstrating iris transillumination and absent platelet dense bodies. In addition, patients with mutations in *HPS4* appear to be at risk for developing pulmonary fibrosis and granulomatous colitis; the relatively severe HPS-4 phenotype is similar to that of HPS-1. In fact, melanocytes from the *HPS4*-deficient light ear mouse are not only deficient in the HPS4 protein, but also lack the HPS1 protein. This points to a possible interaction of the two proteins, as well as their functioning in the same pathway. It also explains the similar phenotypes of HPS-1 and HPS-4 patients.

Short-Chain Acyl-Coenzyme A Dehydrogenase Deficiency: Expanding the Phenotype. *E.A. Crombez¹, M. Fox¹, N. Gregersen², E. Vilain^{1,3}.* 1) Pediatrics, UCLA Medical Center, Los Angeles, CA; 2) Molecular Medicine, Aarhus University Hospital, Denmark; 3) Human Genetics, UCLA Medical Center, Los Angeles, CA.

Short-chain acyl-coenzyme A dehydrogenase [SCAD] is found in the mitochondrial matrix and is involved in the beta-oxidation of saturated fatty acids. SCAD deficiency, once thought of as rare, is becoming increasingly more often diagnosed. SCAD deficiency typically presents in the neonatal or newborn period with metabolic acidosis, failure to thrive, developmental delay, and seizures. There are only nineteen reported cases confirmed by enzyme assay in cultured skin fibroblasts. Here, we present two additional cases of confirmed SCAD deficiency that expand the clinical phenotype. Both patients presented in the newborn period with respiratory distress and dysmorphic features. Both patients later developed cyclic vomiting. In addition, the first patient had: hypotonia, low set ears, high arched palate, broad nasal bridge, pectus excavatum, symmetrical hand anomalies, and malformed feet. The second patient had: facial asymmetry, deformation of the limbs with contractures, adducted thumbs, a defect of the abdominal wall, bilateral cryptorchidism, bilateral hip dislocations, and soft tissue webbing in the upper extremities. Laboratory evaluation in both patients revealed elevated ethylmalonic and methylsuccinic acid in the urine. Acylcarnitine profile showed elevated C4 compounds. In the first patient DNA analysis revealed the patient to be homozygous for the SCAD gene mutation 319C>T which changes the amino acid arginine to cysteine. In the second patient DNA analysis revealed the patient to be homozygous for the SCAD gene variation 625G>A which results in a glycine to serine amino acid change. These are both known susceptibility SCAD gene variations. The diagnosis of SCAD deficiency was confirmed by enzyme assay in cultured skin fibroblasts in both patients. We conclude that SCAD deficiency may present with congenital anomalies and that SCAD may play an important role during development. This expansion of the clinical phenotype of SCAD deficiency will also help identify new patients with this disorder.

Loss of vision in MPS VI is a consequence of increased intracranial pressure. *J.T. Goodrich¹, P. Harmatz², P.A. Levy¹, R.W. Marion¹.* 1) Children's Hospital at Montefiore/ A. Einstein Col of Med, Bronx, NY; 2) Oakland Childrens' Hospital, Oakland, CA.

Caused by a deficiency of N-acetylgalactosamine 4-sulfatase, Maroteaux-Lamy syndrome (MPS VI) is a rare, progressive autosomal recessively inherited disorder. Of the many clinical features associated with MPS VI, the sudden onset of permanent vision loss is one of the most devastating. The cause of this vision loss is not fully understood: theories include the effect of chronically increased intracranial pressure (IICP) and infiltration of the optic nerve with GAGs. We are currently following three patients with MPS VI who are blind. The youngest of these patients, KG, was diagnosed with MPS VI at birth, because of a positive family history. At 4 years of age, he presented with nystagmus; over the course of the next three weeks, he lost all vision in both eyes. Examinations by an ophthalmologist and neuro-ophthalmologist failed to identify papilledema or any other significant ocular pathology. A CT scan and MRI showed findings consistent with MPS VI, but no apparent signs of IICP were seen. A lumbar puncture performed under general anesthesia revealed a markedly elevated opening pressure (>500 mm H₂O). Based on this finding, a ventriculoperitoneal shunt was placed. Following the procedure, KG has regained some vision. Although no cure for MPS VI exists, one of us (PH), in collaboration with BioMarin, is currently involved in an enzyme replacement trial using Aryplase (TM), a specific form of recombinant N-acetylgalactosamine 4-sulfatase. With a possible treatment close at hand, attempts to prevent patients from developing the most disabling manifestations of this condition are of the utmost importance. Our experience strongly suggests that blindness in MPS VI is caused by IICP that may not be detectable using standard exams and imaging studies. Patients with this condition require lumbar puncture for correct diagnosis. We urge that this course of action be taken in any patient with MPS VI showing signs or symptoms of IICP, even when exams and imaging studies reveal "normal" findings.

Aromatic L-aminoacid decarboxylase (AADC) deficiency: Unusual neonatal presentation and new findings in organic acid analysis (OA). *J.E. Abdenur¹, N.G. Abeling², A.C. van Cruchten², N. Specola¹, L. Jorge¹, A.B. Schenone¹, N.A. Chamoles¹.* 1) FESEN, Buenos Aires, Argentina; 2) Academic Medical Center, University of Amsterdam, The Netherlands.

AADC deficiency is a rare neurotransmitter defect leading to a combined deficiency of catecholamines and serotonin. Patients are usually detected in infancy due to developmental delay, hypotonia and extrapyramidal movements. The diagnosis is based on abnormal neurotransmitter pattern in CSF and reduced AADC activity in plasma. An elevation of vanillic acid (VLA) has been described in a few patients as the only abnormality detected in OA. We report a new patient who presented in the neonatal period with lethargy, hypotonia, metabolic acidosis and recurrent hypoglycemia. An inborn error of intermediate metabolism was suspected. Ammonia, lactic acid and acylcarinitines were normal and urine OA (GC/MS) showed a small increase of vanillic acid. The presumptive diagnosis of AADC deficiency was established. The patient was lost to follow-up until the age of 8 months, when he presented with dystonia, abnormal movements, oculogyric crises and hypothermia. Samples of CSF, plasma and urine were obtained at that time. Repeat OA showed not only increased levels of VLA, but also increased vanilpyruvic (VPA), acetyl-vanilalanine (AVA) and N-acetyl-tyrosine (NAT). No significant differences were found with and without ethoximation. Neurotransmitter analysis in CSF showed a marked increase of vanilalanine (1,200 nmol/l, nl up to 100) with decreased levels of 5-hydroxy-indolacetic acid (5 nmol/l, normal 152-462), homovanillic acid (83 nmol/l, normal 302-845) and methoxy-hydroxy-phenyl-glycol (5 nmol/l, normal 51-112). AADC activity in plasma was nearly undetectable. As reported in other patients, the urinary profile of biogenic amines was consistent with the production of dopamine in the kidney. We postulate that that VPA and AVA could originate from vanilalanine (through a transaminase and an acetylase respectively), while NAT could originate from tyrosine through an AA acetylase. This report expands the clinical presentation of AADC deficiency and adds new markers of the disease for OA analysis.

Cloning and Characterization of the Human N-acetylglutamate Synthase Gene. *L.M. Caldovic¹, H. Morizono¹, R. Gallegos², M.H. Malamy², M. Tuchman¹*. 1) Center for Genetic Medicine, CNMC, Washington, DC; 2) Department of Microbiology, Tufts University, Boston, MA.

N-acetylglutamate synthase (NAGS, E.C. 2.3.1.1) is a mitochondrial enzyme that catalyzes the formation of the N-acetylglutamate (NAG) from glutamate and acetyl-CoA. NAG is an essential allosteric activator of carbamylphosphate synthetase I (CPSI), the first enzyme of the urea cycle. NAGS is thought to regulate ureagenesis by producing variable amounts of NAG, thus modulating CPSI catalytic activity. Moreover, inherited deficiencies in NAGS have been associated with hyperammonemia, presumably due to the loss of CPSI activity. The gene encoding human NAGS was identified and cloned based on its similarity to mouse NAGS which we cloned previously. The human gene was cloned from a liver cDNA library and the open ORF is 85% identical to mouse NAGS. The deduced amino acid sequence contains a putative mitochondrial targeting signal of approximately 50 amino acids at the N-terminus. The cDNA sequence complements an *argA* (NAGS deficient) *E. coli* strain, confirming that it indeed has NAGS catalytic activity. The NAGS cDNA was aligned to the human genome draft sequence to identify the intron/exon boundaries and to determine its chromosomal location. The gene has 7 exons, is approximately 4kb long and maps to chromosome 17 band q21.31. Primers were designed to amplify all NAGS exons and their flanking sequences from genomic DNA of patients with possible NAGS deficiency. These patients have hyperammonemia with the clinical and biochemical characteristics of CPSI deficiency, but show normal CPSI enzyme activity in the liver. We are currently screening these patients for mutations in the NAGS gene. Northern blot analysis was used to examine the size and expression pattern of the NAGS transcript. The gene is expressed in the liver and intestine as a 2.3 kb message. We are currently overexpressing His-tagged versions of the NAGS preprotein and putative mature protein in *E. coli* and purifying them using a nickel affinity column. The purified proteins will be used for biochemical characterization and determination of 3-D structure.

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EUROGLYCAN: A systematic approach towards the understanding, diagnosis and treatment of CDG, a novel group of inborn metabolic disorders caused by defects of glycosylation. *M. Aebi¹, E.G. Berger¹, P. Briones¹, K. von Figura¹, T. Hennet¹, J. Jaeken¹, C. Körner¹, G. Matthijs^{1,2}, E. Van Schaftingen¹, M.A. Vilaseca¹, R. Wevers¹, B. Winchester¹.* 1) Participants of the EUROGLYCAN Network; 2) Co-ordinator (Center for Human Genetics, University of Leuven, Leuven, Belgium).

EUROGLYCAN is a European network for basic and clinical research into congenital disorders of glycosylation (CDG), a rapidly expanding group of metabolic diseases.

The major aims are to 1) promote early diagnosis by offering free diagnostic work-up of new cases; 2) collate patient information, useful for the identification of novel types; 3) raise awareness; and 4) establish a patient group large enough for systematic investigations and clinical trials. To fulfil these aims, a database was created that is maintained by the reference centre (<http://www.kuleuven.ac.be/med/cdg>). National referral centres have been established for the recruitment of patient samples. These centres offer first-line diagnostic services, and provide an interface between the network and the referring clinician. Unsolved cases are dispatched by the reference centre to the expert laboratories within the network for systematic analysis. Up to date, the partners of the network were involved in the identification and elucidation of 10 of the 11 known types of CDG-I and CDG-II, and a plethora of unsolved cases are under investigation.

The ultimate goal of the network is to put a precise diagnosis on all cases of CDG, to promote fundamental research in the field and to extend the diagnostic and therapeutic tools.

The project is funded by grant QLG1-CT2000-00047 of the European Commission.

Acute severe metabolic decompensation in Maple Syrup Disease can be optimally treated without dialysis by utilizing high caloric nutrition containing branched chain-free amino acids: 11 year experience with 52 episodes.

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The goal of therapy in acutely ill patients with Maple Syrup Urine Disease (MSUD) is rapid reduction of plasma branched chain amino acid (BCAA) and ketoacid levels, and resolution of acute signs and symptoms of metabolic decompensation. We assessed the efficacy of primary nutritional therapy with parenteral and/or enteral BCAA-free solution or formula in 52 treatment episodes in 15 patients with acute decompensation of MSUD. Patients were multi-ethnic with ages ranging from 6 days to 14.5 years with 4 episodes involving neonates. Patients experienced acute onset of one or more of the following at presentation: lethargy, coma, seizures, ataxia, emesis or ketoacidosis. Pre-treatment leucine levels were $> 800\text{mmol/L}$ (range 801-3944; mean 1404.82). In addition to nutritional therapy, correction of acidosis, supplementation with valine and/or isoleucine (to correct deficiencies and promote protein accretion), and insulin therapy (to overcome insulin resistant states) were instituted as needed. Dialysis was not performed during any of these episodes. In each episode there was a resolution of acute signs and symptoms of decompensation and ketoacidosis. Plasma leucine levels were reduced to $<500\text{ mmol/L}$ by 72 hr in 42 episodes, and by 120 hr after starting treatment in all episodes. No therapy-related complications were encountered. Conclusions: BCAA-restricted parenteral and/or enteral nutrition therapy can effectively improve the clinical and laboratory abnormalities in the majority of patients with MSUD with metabolic decompensation by reducing plasma BCAA levels, and inducing protein accretion and promoting an anabolic state. The need for dialysis, which is effective but invasive and often associated with complications, was obviated in this cohort.

Improved molecular diagnosis of Canavan Disease by multiplex ligation-dependent probe amplification (MLPA) and sequence analysis: identification of novel mutations. A. Errami^{1,2}, G.S. Salomons³, S.J.M. van Dooren³, J.J.P. Gille¹, G. Pals¹, J.P. Schouten², O. Elpeleg⁴, C. Jakobs¹. 1) Clinical Genetics, VU University Medical Center, Amsterdam, Netherlands; 2) MRC-Holland, Amsterdam, Netherlands; 3) Clinical Chemistry, VU University Medical Center, Amsterdam, Netherlands; 4) Metabolic Disease Unit, Shaare-Zedek Medical Center, Jerusalem, Israel.

Canavan disease, an autosomal recessive neurodegenerative disorder, affecting cerebral white matter, is caused by a deficiency of aspartoacylase (ASPA; MIM 271900). The biochemical hallmark is an increased level of N-acetylaspartic acid in body fluids. The identification of the molecular basis for this defect is of pivotal importance for prenatal diagnosis and carrier detection. We have set up the molecular analysis for Canavan disease not only by sequence analysis at the DNA and RNA level, but also developed a multiplex ligation-dependent probe amplification (MLPA) test. This test allows the identification of deletions or duplications of one or more exons of the ASPA gene. Methods: A BLAST (www.ncbi.nlm.nih.gov/) search with the ASPA mRNA (Genbank: AC025125) against the htgs database identified a contig that contained the almost complete ASPA gene (Genbank: NM_000049). We designed PCR primers for all 6 exons. Purified PCR products were directly sequenced and aligned with the ASPA gene. Furthermore, for the MLPA test we designed and synthesized probes specific for each of the exons. After ligation, which depends on the presence of target sequences in the DNA sample, the probes were amplified by PCR. The PCR fragments were analyzed by capillary electrophoresis. Results: In 11 unrelated patients we identified two mutant alleles per patient, including 3 deletions that comprises one or more exons of the ASPA gene and four other novel mutations. In conclusion: DNA based sequence analysis of the ASPA gene allows the identification of mutations that remained undetected by RNA based sequence analysis. The MLPA test proved the presence of deletions. Furthermore homozygosity could be confirmed by this new method.

Orthotopic Liver Transplantation (OLT) in Ornithine Transcarbamylase Deficiency (OTCD) carriers. M.J.

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OTCD is the most common inherited urea cycle disorder. The gene is X-linked causing fatal hyperammonemia in affected males and widely variable clinical severity in female carriers. Despite protein restriction and the use of sodium benzoate and phenylbutyrate, such patients continue to experience recurrent attacks of severe hyperammonemia associated with significant morbidity and mortality. OLT is the only currently available curative procedure as it corrects hyperammonemia, halts progress of disease and improves quality of life. The neurological outcome after transplantation correlates closely with the status prior to transplantation. The decision to perform definitive OLT in carrier females is still controversial and depends on several factors including: phenotypic severity, failure of medical therapy, availability of expertise in the management of acute hyperammonemia, and social factors. Here, we report on two unrelated symptomatic OTCD female carriers corrected with OLT at 14-years of age. Patient 1 (African American) with OTCD diagnosed at 7-years of age. Her OTC enzyme activity was low at 1.3 umol/gm liver/min(80.6+/-5.5). She carried a deleterious OTC mutation (G195R) in exon 6. Patient 2 (caucasian) was diagnosed at 10 years of age. Her OTC enzyme activity was also reduced at 304 umol/gm liver/hr(1500-9000). Using SSCP analysis, no OTC-DNA mutation was found. During acute hyperammonemia, H-MRS of the brain showed elevated glutamine and decreased choline and myoinositol. Basal ganglia calcification in addition to focal seizure activity developed in patient 2. Post-operative immune suppression regimen (prednisone, mycophenolate mofetil, FK506) was well tolerated. Patient 1 underwent biliary reconstruction due to a bile leak. At 6 months post OLT, their hyperammonemia is corrected and neurological status stabilized. No recurrence of seizure activity (patient 2) was reported. We suggest that clear guidelines regarding indications for OLT in OTCD carrier females be developed to avoid the significant morbidity and mortality seen in this metabolic disorder.

Post-partum "psychosis" in mild argininosuccinate synthetase deficiency. *G.M. Enns¹, W.E. O'Brien², K. Kobayashi³, H. Shinzawa⁴, K. Hart¹, H.Z. Gao³, A. Tabata³, T. Saheki³.* 1) Dept Ped, Div Medical Genetics, Stanford Univ, Stanford, CA; 2) Dept Mol Hum Genet, Baylor College of Medicine, Houston, TX; 3) Dept Biochemistry, Kagoshima Univ, Kagoshima, Japan; 4) Dept Internal Med, Public Okitama General Hospital, Yamagata, Japan.

Although underlying ornithine transcarbamylase (OTC) deficiency may be unmasked for the first time after childbirth, reports of other urea cycle disorders presenting for the first time in the post-partum period are rare. We describe two patients, without a past history suggestive of a metabolic disorder, who were diagnosed with argininosuccinate synthetase (AS) deficiency after experiencing neurologic complications post-partum. Patient 1 developed speech and gait abnormalities, which resolved spontaneously, after the birth of her first child. She had memory loss and aggressive behavior and was placed in a psychiatric ward for "post-partum depression/psychosis" after the birth of her second child. She progressed to a coma and was diagnosed with acute fatty liver of pregnancy (AFLP) when mild hyperammonemia ($\text{NH}_3=95\text{mM}$) was found. Post-partum confusion, lethargy, and hyperammonemia ($\text{NH}_3=433\text{mM}$) were present after her third pregnancy. She was again initially diagnosed as having AFLP. Metabolic investigations were performed at that time and showed elevated citrulline. Argininosuccinate lyase activity was normal. Fibroblast AS activity was undetectable and a ^{14}C -citrulline/ ^3H -leucine incorporation assay confirmed the enzymatic defect. She was found to have two novel mutations in the AS gene present in a compound heterozygous state in exon 13 (K310R, delG892). Patient 2 had hyperammonemia at age 25 years at the start of breast feeding following delivery and has been reported previously (Am J Hum Genet 55:1103). DNA analysis detected both mutations in the AS gene (A118T, ins37b/Exons 15 and 16). Urea cycle disorders other than OTC deficiency may present for the first time with neurologic and psychiatric symptoms post-partum and may be an underappreciated cause of such complications. We recommend obtaining metabolic labs immediately in any woman with neurologic or severe psychiatric symptoms in the post-partum period.

Thiamine-responsive pyruvate dehydrogenase deficiency in two patients caused by a point mutation (F205L and L216F) within the thiamine pyrophosphate binding region. *E. Naito¹, M. Ito¹, I. Yokota¹, T. Saijo¹, J. Matsuda¹, Y. Ogawa¹, S. Kitamura¹, E. Takada², Y. Horii³, Y. Kuroda¹.* 1) Department of Pediatrics, School of Medicine, University of Tokushima, Tokushima, Japan; 2) Department of Pediatrics, Saitama Medical Center, Saitama Medical School, Saitama, Japan; 3) Department of Pediatrics, Yamashiro Hospital, Kyoto, Japan.

The human pyruvate dehydrogenase complex (PDHC) catalyzes the thiamine-dependent decarboxylation of pyruvate. Thiamine treatment is very effective for some patients with PDHC deficiency. Among these patients, five mutations of the pyruvate dehydrogenase (E1) α subunit have been published previously: H44R, R88S, G89S, R263G, and V389fs. All five mutations are in a region outside the thiamine pyrophosphate (TPP)-binding region of the E1 α subunit. We report the biochemical and molecular analysis of two patients with clinically thiamine-responsive lactic acidemia. The PDHC activity was assayed using two different concentrations of TPP. These two patients displayed very low PDHC activity in the presence of a low (1×10^{-4} mM) TPP concentration, but their PDHC activity significantly increased at a high (0.4 mM) TPP concentration. Especially, the PDHC activity of patient 2 increased to within the normal range. Therefore, in order to diagnose this type of thiamine-responsive PDHC deficiency and to prevent a diagnostic error, it is necessary to measure the activity of PDHC in the presence of a low (1×10^{-4} mM) as well as a high TPP concentration. Thus the PDHC deficiency in these two patients was due to a decreased affinity of PDHC for TPP. Treatment of both patients with thiamine resulted in a reduction in the serum lactate concentration and clinical improvement, suggesting that these two patients have a thiamine-responsive PDHC deficiency. The DNA sequence of these two male patients' X-linked E1 α subunit revealed a point mutation (F205L and L216F) within the TPP-binding region in exon 7.

A transgenic neuronal cell model for maple syrup urine disease. *A.L. Kasinski, D.J. Danner.* Department of Human Genetics, Emory University School of Medicine, Atlanta, GA.

Maple syrup urine disease [MSUD] is an autosomal recessive inherited metabolic disorder characterized by an extreme rise of the branched-chain amino acids [BCAAs] and their corresponding ketoacids in body tissues and fluids. Mutations in the gene for one of three of the branched-chain α -ketoacid dehydrogenase [BCKD] proteins are causative of MSUD. Transamination by branched-chain amino transferases plays an important role in nitrogen shuttling and metabolism of the excitatory neurotransmitter glutamate in the brain. Excitotoxicity is a contributing element in the neuropathy of MSUD, which may explain the convulsions and cerebral edema characteristic of these patients. To investigate the mechanism for toxicity, we prepared an artificially tet-on regulated transgenic neuronal cell model that over-expresses the murine BCKD-kinase in PC12 cells. BCKD-kinase activity results in inhibition of BCKD. Southern blots verified the presence of transgene in clonally selected cells. The cloned cells retained the ability to respond to nerve growth factor. Addition of doxycycline [DOX] increased BCKD-kinase expression with a concomitant increase in phosphorylated E1 α and a marked decrease in activity-state of BCKD. Preliminary studies show an increase in cell death in doxycycline induced clones when the cells are treated with BCAA and/or branched-chain ketoacid derivatives. The mechanisms for cell death are being investigated to provide a better understanding of the neuropathology seen in MSUD.

Arginase induction by sodium phenylbutyrate in mouse tissues and human cell lines. *R.M. Kern¹, Z. Yang³, P.S. Kim³, W.W. Grody^{1, 3, 4, 5}, S.D. Cederbaum^{1, 2, 4, 5}, R.K. Iyer^{1, 3}.* 1) Mental Retardation Research Center; 2) Depts. of Psychiatry; 3) Pathology; 4) Human Genetics; 5) Pediatrics, David Geffen School of Medicine at UCLA, Los Angeles, CA.

Hyperargininemia is a urea cycle metabolic disorder caused by genetic mutations in arginase I (AI) resulting in elevated blood arginine and ammonia levels. Sodium phenylacetate and its precursor sodium phenylbutyrate (NaPB) have been used to lower ammonia, conjugating glutamine to produce phenylacetylglutamine which is excreted in urine. The elevated arginine levels induce the second arginase (AII) in patient kidney and kidney tissue culture. It has been shown that NaPB increases expression of some target genes and we tested for arginase induction.

Eight 9 week old male mice fed on chow containing 7.5 gm NaPB/kg rodent chow and drank water with 10 gm NaPB/liter, and four control mice had a normal diet. After one week all mice were sacrificed. The arginase specific activities for control and NaPB mice respectively were 38.2 and 59.4 U/mg in liver, 0.33 and 0.42 U/mg in kidney, and 0.29 and 1.19 U/mg in brain. Immunoprecipitation of arginase in each tissue with AI and AII antibodies showed the activity induced by NaPB is mostly AI. AII may also be induced but not as significantly. AI accounts for the four-fold increased activity in brain.

In some cell lines, NaPB increased arginase activity up to 5 fold depending on dose (1-5 mM) and exposure time (2-5 days); control and NaPB activities respectively are: erythroleukemia, HEL, 0.06 and 0.31 U/mg, and K562, 0.46 and 1.74 U/mg; embryonic kidney, HEK293, 1.98 and 3.58 U/mg; breast adenocarcinoma, MDA-MB-468, 1.11 and 4.06 U/mg; and prostate adenocarcinoma, PC-3, 0.55 and 3.20 U/mg. In MDA-MB-468 and HEK most, but not all, of the induced activity is AI.

These studies suggest that NaPB may induce AI when used to treat urea cycle disorders. It is relatively less useful in AI deficiency, although it could have some effect in those with missense mutations. It is tempting to speculate that the induction in brain particularly may be due to the glutamine depletion that occurs with the NaPB administration.

The frequency of short-chain acyl-CoA dehydrogenase (SCAD) gene variants in the U.S. population and correlation with the C4-acylcarnitine (C4-AC) concentration in newborn blood spots. *D. Matern, A. Tauscher, K. Kruckeberg, P. Rinaldo, N. Nagan.* Division of Laboratory Genetics, Mayo Clinic, Rochester, MN.

SCAD deficiency is clinically heterogeneous with patients described as being basically asymptomatic while others had fatal outcomes. Two SCAD gene variants (511C>T; 625G>A) are known. While they cause non-conserved amino acid substitutions that alter the structural and catalytic enzymatic properties, they are neither true disease causing mutations nor polymorphisms but mutations that confer disease susceptibility. Previous studies found these gene variants to be common in the Northern European population (*Pediatr Res* 2001;49:18-23). We aimed to determine the frequency of these variants in the U.S. population and whether their presence correlates with elevated C4-AC concentrations in newborn blood spots. Methods: DNA was purified from blood spots collected prospectively from 612 newborns. The presence of SCAD gene variants was determined using LightCycler technology. C4-AC concentrations in each blood spot were measured by tandem mass spectrometry. Results: 5.2% of all newborns were homozygous for 625G>A, 35.1% were heterozygous and 59.7% were wild type homozygous for this variant. With respect to the 511C>T variant, 93.4% of the newborns were wild-type, 0.1% homozygous and 6.5% heterozygous. 7.4% of the study population were homozygous or compound heterozygous for these variants. The percentage of newborns homozygous for 625A differed among Hispanics (12%) and African-Americans (2%) compared to the predominantly white study population (6%). C4-AC concentrations in newborn blood spots correlated ($p<0.001$) with homozygosity for the 625A variant. Discussion: The frequency of the 625G>A SCAD gene variant was found to be more frequent than 511C>T. While homozygosity for 625G>A correlates with higher C4-AC concentrations than observed in the wild type population, none of the observed genotypes causes C4-AC concentrations strongly suggestive of SCAD deficiency. To further elucidate the clinical significance of these findings, newborn screening blood spots of patients with genotypes involving only these gene variants and biochemically confirmed SCAD deficiency should be analyzed.

Genotype Determinants of Phenotype in Erythropoietic Protoporphyria. *H. Risheg, F. Chen, J.R. Bloomer.*
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Excessive accumulation of protoporphyrin in erythropoietic protoporphyria (EPP) results from a deficiency in the activity of ferrochelatase (FECH), which catalyzes the final step in heme biosynthesis. This causes the clinical manifestations of EPP, lifelong photosensitivity and in some, liver disease necessitating transplantation. FECH DNA analysis from our lab showed 38 EPP individuals from 24 families to be heterozygous for a variety of FECH mutations, which did not satisfactorily explain phenotype. It was also shown that an intron polymorphism (IVS3-48c) in the FECH gene was associated with frequent formation of aberrantly spliced FECH mRNA, which was rapidly degraded (Gouya et al, Nat Genet 30:2002). **AIM:** Define further the genotype-phenotype relationship in EPP, focusing on the role of this polymorphism. **METHODS AND RESULTS:** The IVS3-48c was examined by sequencing of intron 3 and correlated with disease severity. This revealed 93% of 30 symptomatic individuals with FECH mutations (30 photosensitive; 14 with liver disease) to be heterozygous for the IVS3-48c, whereas 8 asymptomatic individuals with FECH mutations were homozygous for the predominant IVS3-48t polymorphism. Two of 40 controls (5%) were heterozygous for the IVS3-48c. Haplotype analysis was used to trace the mutant and non-mutant FECH alleles in families with asymptomatic and symptomatic individuals. Allele specific oligonucleotides were used to assess the level of normal FECH mRNA by relative quantitative RT-PCR. Results showed symptomatic family members to share the same haplotype for the non-mutant FECH allele, containing the IVS3-48c. Conversely, asymptomatic family members carried the IVS3-48t in their non-mutant allele. Levels of normal FECH mRNA were significantly lower in cultured lymphoblasts of individuals with the IVS3-48c. **CONCLUSIONS:** Symptomatic disease in most EPP patients is explained by the inheritance of a mutation in one FECH allele which causes a structural alteration in the FECH protein that abolishes enzyme activity, together with a low expressing non-mutant FECH allele which is caused by the IVS3-48c. The findings have significant implications for counseling in EPP, and are relevant in other disorders with variable disease expression.

The mtDNA G-A mutation at nt 13513, previously reported in MELAS syndrome, is a frequent cause of Leigh disease. *M. CHOL*¹, *S. LEBON*¹, *P. BENIT*¹, *D. CHRETIEN*¹, *P. DE LONLAY*¹, *V. CORMIER*^{1,2}, *P. RUSTIN*¹, *A. ROTIG*¹, *A. MUNNICH*^{1,2}. 1) INSERM U393, Hopital Necker, Paris, France; 2) Departement de Genetique, Hopital Necker, Paris, France.

Leigh syndrome is characterized by subacute necrotizing encephalomyopathy and is often associated with mitochondrial respiratory chain deficiency. This disease is genetically heterogeneous, as mutations in both mitochondrial (mt) and nuclear genes have been reported in various patients. Here we report on three patients presenting Leigh syndrome associated with a complex I deficiency and harboring a G13513A transition in mtDNA ND5 gene. This mutation was found to be heteroplasmic in all tissues tested, a high percentage of mutant mtDNA being observed in muscle DNA. The asymptomatic maternal relatives presented lower levels of G13513A mutant mtDNA in blood leukocytes. This mutation affects an evolutionary conserved amino acid (D393N). These results suggest that the G13513A transition is likely to be pathogenic. Moreover, this mutation has been previously reported in patients with MELAS or LHON/MELAS syndrome, again demonstrating the phenotypic heterogeneity of mitochondrial DNA mutations. Interestingly, the G13513A mutation seems to represent a frequent cause of Leigh disease as it was found in 21% (3/14) of our patients presenting with Leigh syndrome and complex I deficiency. We therefore suggest that the G13513A mutation in the mtDNA ND5 gene should be systematically screened in Leigh patients and their relatives for molecular diagnosis and genetic counseling.

Mistargeting of mitochondrial NDUFV2 mRNA results in complex I deficiency and causes early onset hypertrophic cardiomyopathy. P. BENIT¹, R. BEUGNOT², D. CHRETIEN¹, P. de LONLAY¹, JP. ISSARTEL², S. KERSCHER³, P. RUSTIN¹, A. MUNNICH¹, A. ROTIG¹, M. CORRAL-DEBRINSKI⁴. 1) Dept Genetics, INSERM U393, Hosp Necker, Paris, France; 2) CEA-DBMS, CEA Grenoble, 17 rue des Martyrs, 38054 Grenoble, Cedex 9, France; 3) Universitätsklinikum Frankfurt, Institut für Biochemie I, ZBC, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany; 4) CNRS URA 1302, Ecole Normale Supérieure, 46 rue d'Ulm, 7505 Paris, France.

Most of the mitochondrial proteins are encoded by nuclear genes, synthesized in the cytosol with a N-terminal mitochondrial targeting signal, which is subsequently cleaved within the mitochondria to give rise to the mature form of the protein. However, a number of mitochondrial proteins are co-translationally imported. Here we show that NDUFV2 mRNAs, which encodes a respiratory chain complex I subunit, as well as several mRNAs encoding mitochondrial proteins, are clustered around the mitochondria in human cultured skin fibroblasts and/or HeLa cells, suggesting a co-translation translocation of these proteins. Studying nuclear genes encoding complex I subunits in a cohort of 42 patients with complex I deficiency, we identified a deletion of NDUFV2 exon 2 in two patients presenting an early onset hypertrophic cardiomyopathy. The deletion of exon 2 which encodes the Ndufv2 targeting sequence resulted in a roughly 50% reduction of the Ndufv2 protein in patient's fibroblast mitochondria compared to control. Accordingly, a 50% decrease of complex I activity was detected in patient's skeletal muscle, heart and fibroblasts. Decreased protein content in patient fibroblasts correlated with an abnormal sub-cellular localization of NDUFV2 mRNAs which were present in free-polysomes rather than in mitochondria-bound polysomes. The quantitative comparison of residual NDUFV2 mRNA bound to mitochondria, residual amount of mitochondrial Ndufv2 protein, and residual enzyme activity indicate that mistargeting of NDUFV2 mRNA probably accounts for complex I deficiency. This suggests that abnormal mRNA distribution could represent a novel mechanism of complex I deficiency in human.

Cell complementation using Genebridge 4 human-rodent hybrids for physical mapping of mitochondrial respiratory chain deficiency genes. *P. de Lonlay*¹, *C. Mugnier*², *D. Sanlaville*³, *V. Geromel*¹, *P. Benit*¹, *D. Chretien*¹, *N. Kadhom*¹, *K. Chantrel*¹, *S. Saker*⁴, *G. Gyapay*⁴, *S. Romana*³, *A. Munnich*¹, *P. Rustin*¹, *A. Rotig*¹. 1) INSERM U393, Hosp Necker-Enfants Malades, Paris Cedex, France; 2) Service Informatique, Hosp Necker-Enfants Malades, Paris Cedex, France; 3) Service de Cytogenetique, Hopital Necker-Enfants Malades, Paris; 4) Genethon, Evry, France.

Mapping and identification of genes responsible for mitochondrial disorders is a difficult endeavour due to the large number of genes either encoding subunits of the respiratory chain (RC) or involved in respiratory chain assembly. We have developed a functional complementation approach for mapping mitochondrial disease genes by combining the use of a highly selective medium for respiratory-competent cells and microcell-mediated transfer of human genetic material in RC-deficient fibroblasts. We first showed that the absence of any ose in the culture medium resulted in a rapid death of RC-deficient fibroblasts. This highly selective medium could therefore be used to rapidly screen for restoration of RC capacity by genetic transfer. Four cell lines of patients with complex I, II or I+IV deficiency were fused with microcells prepared from human:rodent Genebridge 4 panel of whole genome radiation hybrids allowing to map the disease-causing genes to Xq22, 12p12, 21pter and 7p22. These candidate regions were therefore limited to 4 to 30 Mb. This approach allowed us to physically map the disease gene in four sporadic cases. These genetic intervals are similar to that obtained by genetic linkage analyse in large informative families.

Mitochondrial localization of PANK2 and discovery of a common polymorphism that prevents it. *M.A. Johnson¹, Y.M. Kuo², S.K. Westaway¹, S.M. Parker¹, J. Gitschier², S.J. Hayflick¹*. 1) Departments of Molecular and Medical Genetics, Pediatrics and Neurology, School of Medicine, Oregon Health & Science University, Portland, OR; 2) Howard Hughes Medical Institute and Departments of Medicine and Pediatrics, University of California, San Francisco, CA.

Recently we reported that mutations in *PANK2*, one of the four human pantothenate kinase genes, causes pantothenate kinase associated neurodegeneration (PKAN, formerly Hallervorden-Spatz syndrome). Though only cytosolic pantothenate kinase enzymatic activity has been described previously, a mitochondrial targeting sequence (MTS) is uniquely predicted at the amino-terminus of *PANK2*. We transfected full-length human *PANK2* cDNA bearing a carboxy-terminal *myc* tag and observed mitochondrial localization of *PANK2* by immunofluorescence. Immunohistochemistry with antibodies against *PANK2* confirmed localization of the endogenous protein to mitochondria. Mitochondrial localization provides insight into why defects in *PANK2*, expressed ubiquitously, lead specifically to neurodegeneration.

Interestingly, *PANK2* begins with an unconventional CTG translation initiation codon, and we observed that 4% of control chromosomes harbor a CAG codon variant. *In vitro* mutation of the CTG codon to CAG led to cytosolic localization of *PANK2* in transfection experiments, presumably due to use of the methionine initiation codon in the middle of the MTS. This observation suggests that the CAG polymorphism has a likely functional consequence of improper *PANK2* localization. We are currently asking whether this sequence variation may represent a susceptibility allele to common neurodegenerative disorders like Parkinson disease or isolated pigmentary retinopathy.

Mitochondrial porin deficient mice: a murine model of Mendelian respiratory chain defects and encephalopathy.

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Voltage dependent anion channels (VDACs; porins) are the main pathways for metabolites across the mitochondrial outer membrane and may constitute one component of the permeability transition pore that opens in response to apoptotic signals. Mammals have four VDAC isoforms encoded by three separate genes, each with different electrophysiological behavior. VDAC1(-/-), VDAC3(-/-) and VDAC1/3(-/-) mice have been generated and exhibit abnormalities in spatial and associative learning that resemble the neurological deficits seen in patients with respiratory chain defects. These learning deficits are accompanied by abnormalities of hippocampal synaptic plasticity. Since mitochondrial enzyme assays in skeletal muscle of VDAC1(-/-) animals demonstrate a reduction in cytochrome c oxidase (COX) activity, we sought to determine whether brain tissue from VDAC(-/-) mice exhibits any respiratory chain (RC) defect that could be correlated with cognitive deficits. Mitochondrial RC enzyme assays of brain mitochondria of VDAC1(-/-), VDAC3(-/-) and VDAC1/3(-/-) reveal significant RC defects. There is a marked increase in citrate synthase activity in VDAC1 and VDAC1/3 (-/-) mice, with partial deficiencies of complex I, II, III and IV activity. In the brain mitochondria of VDAC3(-/-) mice there is a marked decrease in COX activity but normal citrate synthase activity. We postulate that the observed RC defects in VDAC deficient mice could be secondary to increased reactive oxygen species or the defective synthesis or assembly of cytochrome c oxidase subunits. Preliminary data suggest that in VDAC1/3(-/-) mice there is an increase in oxidative modification of proteins by reactive oxygen species when compared to wildtype mice and reduced expression of RC proteins in VDAC1(-/-) mice. In summary, VDAC deficient mice exhibit neurological and biochemical deficits seen in patients with RC defects. These data point to a role for VDAC in the modulation of the mitochondrial respiratory chain and we speculate that it is related to abnormal mitochondrial calcium homeostasis and altered gene expression.

Mitochondrial DNA depletion associated with methylmalonic aciduria, and deficiency of 3-methylcrotonyl CoA and propionyl CoA carboxylase. A new disorder of generalized mitochondrial dysfunction? *S. Yano¹, K. Moseley¹, A. Guedalia¹, L. Li¹, J. Lee¹, T.P. Le², R.G. Boles¹.* 1) Div Medical Genetics, Childrens Hospital Los Angeles, Los Angeles, CA; 2) Biochemical Genetics, Pediatrics, University of California San Diego, San Diego, CA.

Mitochondrial DNA (mtDNA) depletion, referring simply to a low mtDNA copy number, is highly heterogenous in terms of associated phenotype (Leigh disease, Alpers syndrome, cardiomyopathy, etc.) and causal genotype (deficiencies of deoxyguanosine kinase, gamma-polymerase, etc.). We report two very similar cases (Pt.1, a 3 y-old Hispanic male; Pt. 2, an unrelated 2 y-old Hispanic female) with mtDNA depletion who were both diagnosed at 5 months of age with failure to thrive, developmental delay, hypotonia, and metabolic acidosis. A diagnosis of methylmalonic aciduria was initially raised in both based upon substantial elevations in urine methylmalonate, 3OHpropionate and methylcitrate by GC/MS, and plasma propionyl- and methylmalonyl-carnitines by MS-MS. Additional elevations found at least once in both children included 3 OHpropionate, 3OHisovalerate, methylmalonate, fumarate, glutarate, malate, pyruvate, 2OHglutarate, isocitrate, citrate, methylcitrate and 2-oxoglutarate. Assay in cultured skin fibroblasts revealed low propionyl CoA and methylcrotonyl CoA carboxylase activities with normal pyruvate carboxylase activity, again in both children. Quadriceps muscle showed focal mitochondrial accumulation and partial complex IV deficiency in patient 1, and abnormal mitochondrial structures with prominent concentric forms and partial deficiencies in complexes I, II/III, and IV in patient 2. Quantitative Southern blot analysis in muscle tissue revealed mtDNA depletion of 24% (Pt1) and 39% (Pt2) to normal controls. Our two patients have multiple mitochondrial dysfunction including defects in mtDNA synthesis/maintenance, and deficiencies in two carboxylases. To our knowledge, there is no obvious connection between the defective pathways in our cases, such as a common co-factor. The primary defect could involve mitochondrial import, biogenesis or membrane integrity, and studies are ongoing to address these possibilities.

Preliminary characterization of a mouse model of diastrophic dysplasia. A. Rossi¹, C. Tiveron², R. Piazza¹, A. Superti-Furga³, L. Tatangelo², S. Della Torre¹, A. Forlino¹, G. Cetta¹. 1) Department of Biochemistry, University of Pavia, Pavia, Italy; 2) Centro Ricerca Sperimentale, Istituto Regina Elena, Rome, Italy; 3) Department of Pediatrics, University of Zurich, Switzerland.

Mutations in the diastrophic dysplasia sulfate transporter (DTDST) gene (SLC26A2) have been associated with a family of recessive chondrodysplasias including, in order of increasing severity, a mild form of multiple epiphyseal dysplasia, diastrophic dysplasia (DTD), atelosteogenesis type 2 and achondrogenesis 1B. The gene encodes for a widely distributed sulfate-chloride antiporter of the cell membrane whose function is crucial for the uptake of inorganic sulfate which is needed for proteoglycan (PG) sulfation. A correlation between the nature of the mutations, the residual function of the sulfate transporter, the degree of PG sulfation and the different clinical phenotypes have been traced. However, to better understand the pathogenetic mechanisms and to explore possible therapeutic avenues a mouse model would be useful. Using homologous recombination in embryonic stem cells (ES AB1 were kindly provided by Dr. A. Bradley, Houston), we have generated a transgenic mouse homozygous for the *ddtst* mutation A386V that we had identified in a patient with classical diastrophic dysplasia (Rossi et al. Hum. Mutat. 17, 159-171, 2001). The neomycine resistance gene in intron 2 was flanked by loxP sequences for possible excision by Cre recombinase.

By clinical inspection, radiography and differential staining of whole mice with Alizarin Red S and Alcian blue, transgenics that are heterozygous for the A386V substitution are phenotypically normal. In contrast, homozygotes grow slowly with an overall shorter skeletal system and reduced body weight compared to normal litter mates. Their movements are impaired, and they show severe thoracic kyphosis and hip dysplasia. Thus, the human DTD mutation introduced in the murine *ddtst* locus produces a skeletal and connective tissue phenotype very reminiscent of human diastrophic dysplasia. Work supported by Telethon-Italy grant D.83 and B.55 (to CT and LT), Italian MURST grant MM05148132-3 and Fondazione Cariplo.

Minimal transcript level changes in cerebellum of a mouse genetic model for Down syndrome. *R.H. Reeves¹, N.G. Saran¹, J. Natale², M. Pletcher¹*. 1) Dept Physiology, P202, Johns Hopkins Univ Sch Med, Baltimore, MD; 2) Children's Hospital, Washington D.C.

Trisomy 21 results in Down Syndrome (DS), the most common genetic cause of mental retardation. Cerebellar volume is disproportionately reduced in the DS brain. Ts65Dn (Ts) mice are at dosage imbalance for about half of the genes on Chr21. They demonstrate significant reduction in cerebellar volume, and histological analysis identified specific changes that correctly predicted alterations in the DS brain (Baxter et al., 2000). These changes are dosage sensitive, with milder affects in the Ts1 mouse which has fewer Chr21-homologous genes at dosage imbalance (see L. Olson et al.).

We compared transcript profiles in 5 Ts mice to those in 5 euploid (Eu) littermates in duplicate on Affymetrix U74A chips (20 hybridizations total). Those probes with a high proportion of "absent" calls were eliminated. The CLUSTER program sorted all experiments (Eisen et al. 1998); we analysed the three (12 chips) in which all duplicates were most closely matched. The resulting trees grouped Ts separately from Eu mice, i.e., gene expression profiles discriminated between the two groups. No one functional category of genes was evident among these. Only 25 of 6601 probes varied by 2-fold or more in either direction, and only 171 (2.6%) showed a 1.5-fold change, half increased and half decreased; nine of the 18 human Chr21 homologs on the chip increased 1.5-fold. 97% were essentially unchanged in transcript level.

The predominant cell type in the cerebellum is the granule cell (gc) neuron - indeed, gc represent 50% of all neurons in the adult brain - thus, we essentially measured gc transcript levels. Despite significant changes in granule cell number and the architecture of the internal granule layer, only subtle differences in expression distinguish Ts from Eu neurons. At the transcript level, those neurons that survive to adulthood are relatively normal, and the gene dosage effects of trisomy may be more pronounced in the developmental processes that produce the adult structure.

Membrane regulation in dysferlin deficiency: Evidence for disrupted membrane signaling and altered remodeling through expression profiling. *D.A. Rao^{1,2}, E. Veszelyovszky¹, P.H. Plotz², E.P. Hoffman¹*. 1) Center for Genetic Medicine Research, Children's National Medical Center, Washington, DC; 2) Arthritis and Rheumatism Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH, Bethesda, MD.

Deficiency of dysferlin, a skeletal muscle plasma membrane protein, causes two recessively inherited myopathies: Limb Girdle Muscular Dystrophy type 2B and Miyoshi Myopathy, a distal myopathy. Both clinical presentations involve slowly progressive muscle weakness with onset in the late teens, and either presentation can result from the same mutation in dysferlin. Dysferlin shares significant homology with *C. elegans* factor FER-1, a protein required for worm sperm vesicle fusion, indicating a role for dysferlin in membrane fusion events, and EM analysis of human dysferlin-deficient muscle reveals numerous membrane abnormalities, including accumulation of subsarcolemmal vesicles. In this study, we used expression profiling to examine the secondary downstream effects of a primary deficiency in dysferlin. We used Affymetrix U95A and MuscleChip microarrays to generate 14 expression profiles from 5 patients with dysferlin deficiency. By comparing these profiles to profiles of normal muscle (22) and of muscle from patients with Duchenne Muscular Dystrophy (26) using both Affymetrix and GeneSpring software (Welch t-tests, $p < 0.05$), we isolated 121 genes with dysregulation specific to dysferlin deficiency. Seventeen of these genes are involved in membrane regulation, with nine genes known to participate directly in the regulation of vesicle formation or trafficking, including RAD, Rab5C, MLL septin-like fusion, and arfaptin1. We have confirmed altered expression of both vesicle trafficking proteins as well as membrane signaling molecules by QMF-RT-PCR and immunohistochemistry, and have observed altered plasma membrane expression and/or localization of a number of proteins important for membrane signaling and stability. Integration of these expression changes into biochemical pathways will reveal cascades driving myofiber pathogenesis in dysferlin deficiency and promote understanding of dysferlin function at the membrane.

Mutation Screening of the Mitochondrial Genome using Denaturing High-Performance Liquid

Chromatography. *J. Christodoulou*^{1,2}, *A. Biggin*¹, *D.R. Thorburn*³, *B. Bennetts*^{1,2}. 1) Western Sydney Genetics Program, Children's Hospital, Westmead, NSW, Australia; 2) Dept of Pediatrics & Child Health, University of Sydney, NSW, Australia; 3) Murdoch Children's Research Institute, Melbourne, Vic, Australia.

Over 170 disease-causing mutations of the mitochondrial genome have now been identified, and the list continues to grow. The diverse clinical presentations do not often provide pointers that the primary defects might involve mitochondrial DNA (mtDNA). As pathogenic mtDNA mutations are mostly heteroplasmic, denaturing high-performance liquid chromatography (DHPLC) could be used to detect these heteroplasmic species and therefore act as a rapid screening test for mtDNA mutations.

The entire mitochondrial genome was amplified by PCR in 40 overlapping regions. In addition, known mtDNA mutants were constructed for each of these regions using a PCR-based site-directed mutagenesis approach. These mutants were used as positive controls and showed a detection limit of 3-10% heteroplasmy (depending on the specific mutation) compared to 40% for conventional sequencing. To further validate the screening test, mtDNA from 17 patients covering 7 different mutations were used to compare mutation detection by DHPLC and conventional sequencing. DHPLC had a sensitivity (probability that the test was positive if a mutation was present) of 88% compared to 82% for sequencing. This increased to 100% sensitivity for DHPLC when excluding the 8993T>G mutation.

DHPLC analysis is therefore a sensitive, rapid and cost-effective method to screen for mutations in the mitochondrial genome.

A new mutation of the apolipoprotein B gene originates the apo B32,4 truncated protein. *O. Guardamagna¹, E. Bugianesi², R. Bonardi², V. Molini¹, C. Bondone¹, A. Bobbio¹, P. Tarugi³.* 1) Dip.di Scienze Pediatriche e dell'Adolescenza; 2) Dip. dell'Apparato Digerente e della Nutrizione-Universita' di Torino; 3) Dipartimento di Scienze Biomediche,Universita' di Modena,Italia.

Background: Familial hypoblipoproteinemia (FHBL) is an autosomal codominant hypocholesterolemia disorder which prevalence is 1:500/1:3000 in western countries. The clinical phenotype is heterogeneous and homozygous patients present malabsorption and neurological degeneration, while heterozygous ones show a milder phenotype. A relationship between heterozygous FHBL and liver steatosis has been demonstrated. The basic defect relies on the apolipoprotein B (apoB) gene mutation. **Aim of the study** consists in correlating an apoB gene mutation with an unexplained steatohepatitis. **Methods:** A 63yrs old patient being affected from steatohepatitis since adulthood was investigated for FCHL. Total cholesterol and triglycerides were analyzed by standard kit, apoB by immunoturbidimetric method. Plasma lipoproteins were separated by ultracentrifugation and apoB analyzed by immunoblotting and electrophoresed on polyacrylamide gel. The apoB gene mutation detection was performed by PCR amplification of the 5' of the exon 26 and sequenced using the Sequencing System (Promega Co., Madison). **Results:** The conclusive diagnosis of FHBL was based on the apoB gene mutation detection. This is a two base (AG) deletion at codon 1464 causing a stop codon (TAA) at position 1470. This mutation was detected for the first time in this patient who carries an apoB truncated protein which size corresponds to 32,4% of the normal apoB 100. **Conclusions:** A new truncated protein and gene mutation related to liver steatohepatitis have been detected. Although a number of apoB gene mutations were previously recognized correlations between apoB and this phenotype is poorly known and possibly misdiagnosed. Furthermore liver steatosis could represent the initial step evolving to steatohepatitis, as the present case demonstrated, to cirrhosis and hepatocarcinoma.

Thiamine responsive megaloblastic anemia presenting as microcytic anaemia: an unusual case with novel mutations. *A.S. Feigenbaum¹, S. Hewson¹, D. Wherret², J. Doyle³, J.S. Wray⁴, B.D. Gelb⁵.* 1) Dept Genetics and Paediatrics; 2) Div of Endocrinology; 3) Div of Haematology/Oncology, Hospital for Sick Children and University of Toronto, ON, Canada; 4) Program in Human Genetics, McMaster University Medical Centre, Hamilton, ON, Canada; 5) Department of Human Genetics, Mount Sinai School of Medicine, New York, USA.

Thiamine responsive megaloblastic anemia (TRMA) is an autosomal recessive condition characterized by megaloblastic anemia, diabetes mellitus and sensorineural deafness caused by mutations in the thiamine transporter protein SLC19A2.

We describe a child of consanguineous first cousin East Indian parents who presented to us at 8 years of age with sensorineural deafness, insulin dependent diabetes mellitus, retinopathy and anemia since an acute febrile illness at age 3 years. Full mitochondrial work-up was normal. Hemoglobin(Hgb)was 41-100 g/L, with hypochromia (MCHC 310g/L), microcytosis, and dimorphic red cells. Hgb electrophoresis, retics, iron, B12 and folate levels were normal. Bone marrow showed sideroblastic anemia with macronormoblastic dyserythropoiesis.

Father had hypochromia, microcytosis and Hgb electrophoresis consistent with b-thalassemia trait; mother's was normal. Molecular analysis of the α -globin gene cluster revealed father is compound heterozygote for the rightward and leftward single α -globin gene deletions ($-\alpha^{3.7}/-\alpha^{4.2}$). The proband is heterozygous for the rightward single α -globin gene deletion ($-\alpha^{3.7}/aa$).

SLC19A2 mutation analysis identified a novel T439P (A1315C)substitution, homozygous in the proband and heterozygous in parents. Therapy with thiamine showed haemoglobin response from 87 to 115g/L in 3 weeks but no improvement in diabetes control over 2 years of therapy.

We report an unusual case of TRMA with microcytosis associated with α -thalassemia trait rather than macrocytic anemia highlighting the difficulties in diagnosis and illustrating that thiamine therapy may improve the anemia but not the diabetes in this rare condition.

Hermansky-Pudlak Syndrome Type-1: Screening Methods, Mutations, and Clinical Outcomes. *C.R. Hermos, M. Huizing, W.A. Gahl.* Heritable Disorders Branch, NICHD, NIH, Bethesda, Maryland.

Hermansky-Pudlak syndrome is an autosomal recessive disorder characterized by oculocutaneous albinism, a platelet storage pool deficiency and, in some patients, lysosomal accumulation of ceroid lipofuscin. HPS is associated with defects in the biosynthesis and/or processing of melanosomes, platelet dense bodies, and lysosomes. Four human genes (HPS1, ADTB3A, HPS3, HPS4) are associated with 4 subtypes of HPS.

We studied the molecular and clinical aspects of the HPS-1 subtype, caused by mutations in the HPS1 gene. A 16-bp duplication in exon 15 causes HPS-1 in 450 patients in northwest Puerto Rico; 13 other HPS1 mutations have been reported in non-Puerto Rican patients. We screened 26 HPS patients for HPS1 defects using northern blotting, SSCP, restriction enzyme analysis and direct sequencing. Six different HPS1 mutations were identified in 6 of the 26 patients. Four new mutations were discovered, including the first HPS1 missense mutation. New mutations include 624delG in exon 6 and 922T>C in exon 8 which appear to preserve RNA translation and 561delC in exon 5 and [1581delA;1594C>A] in exon 14 which result in no RNA by northern blot. The 6 newly diagnosed HPS-1 patients bring to 13 the total number of non-Puerto Rican individuals with HPS-1. One of 6 adult patients developed pulmonary fibrosis, and 2 patients ages 16 and 17 have granulomatous colitis. These complications are also common among Puerto Rican HPS-1 patients but have not arisen in HPS-2 and HPS-3 patients.

We conclude that the diagnosis of HPS-1, available only on molecular grounds, has important prognostic implications. In addition, the diagnosis may soon influence treatment decisions, since a clinical trial has recently shown that the antifibrotic agent, pirfenidone, slows the decline in pulmonary function associated with HPS1 disease. The screening methods employed in this study should help achieve the accurate molecular diagnosis of HPS-1.

Molecular, biochemical and clinical aspects of Smith-Lemli-Opitz syndrome in Cuban patients. *M.J.M.*

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Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive MCA-MR disorder caused by mutations in the 7-dehydrocholesterol reductase (DHCR7) gene. The diagnosis is based on the findings of elevated plasma 7-dehydrocholesterol (7DHC) levels. To date, more than eighty DHCR7 mutations have been reported in SLOS patients.

Cuba is the largest of the Caribbean islands, having a population in excess of eleven million. The Cuban population is predominantly African in origin, with 11% Cuban-African, 37% European Caucasian, and 51% of mixed European Caucasian and African ancestry. The European Caucasians are almost entirely of Spanish origin. Herein we report the molecular, biochemical and clinical aspects of eight SLOS patients from Cuba. These patients are at the mild end of the clinical spectrum. The Kelly-Hennekam severity scores were <20 for six patients, and 21 and 28 for the remaining two patients. The clinical diagnosis was confirmed by the demonstration of elevated plasma 7DHC levels. Nucleotide sequence analysis showed that one patient was homozygous for the T93M missense mutation, and the remaining patients were compound heterozygotes for T93M and other SLOS alleles (IVS8-1G>C, D234Y, V281M, F302L). Therefore, the T93M mutation is a common SLOS allele in the Cuban population.

The T93M mutation confers a relatively mild phenotype with paucity of physical findings associated with moderate to severe developmental and behavioral problems. This mutation was reported previously in a large proportion of Italian patients, raising the possibility that T93M may be a "Mediterranean mutation". Consistent with this view, the T93M mutation in our Cuban, Italian, and Greek SLOS patients is associated with the same haplotype.

Identification of twelve new arylsulfatase A gene mutations in metachromatic leukodystrophy (MLD) patients.

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Metachromatic leukodystrophy (MLD, MIM 250100) is an autosomal recessive, metabolic disease caused by deficiency of arylsulfatase A activity. The estimated gene frequency is approximately 0.5%, corresponding to an incidence of 1 in 40,000 births. ARSA deficiency causes intralysosomal accumulation of cerebroside sulfate in the white matter of the central nervous system and in the peripheral nerves, leading to progressive demyelination and a variety of neurological symptoms. Clinically the disease is heterogeneous and different forms are classified according to the age of onset (infantile, juvenile, adult).

Surveys of MLD patients have identified more than eighty different *ARSA* gene mutations, with two mutations (IVS2+1G>A and P426L) accounting for up to 50% of the mutant alleles in some populations. The remaining MLD mutations are extremely rare or private.

We have investigated more than fifty MLD patients using multiplex ARMS assays to detect the pseudodeficiency (PD) allele and several common MLD mutations (IVS2+1G>A, I179S, P426L). This was followed by comprehensive nucleotide sequencing of the *ARSA* gene to detect rare mutations, and Southern hybridization to detect large deletions. Here we report the identification of eleven new microlesions in the *ARSA* gene: seven missense mutations (P155L, L181Q, C300Y, Y306H, G325S, E329K, Y429S), three frameshift mutations (g.203-204delTG, g.750-756delCCGCCGG, g.2590-2591insCCCC), and one splice donor site mutation (IVS5+1G>A). We also identified a 14.8 kb deletion that removes the entire *ARSA* gene. Molecular characterization of the deletion endpoints is consistent with homologous recombination between Alu repeat elements that flank the *ARSA* gene. Comprehensive mutation detection has facilitated carrier detection and prenatal diagnosis for several at-risk MLD families.

Characterization of Four Novel b-Galactosidase Gene Mutations and A Polymorphism in Taiwanese Patients with GM1-Gangliosidosis. *J.Y. Wu^{1,2}, C.F. Yang³, F.J. Tsai^{1,4}*. 1) Dept Medical Research, China Medical Col Hosp, Taichung, Taiwan; 2) National Genotyping Center at Academia Sinica, Taipei, Taiwan; 3) IBMS, ACademia Sinica, Taipei, Taiwan; 4) Dept Pediatrics, China Medical Col Hosp, Taichung, Taiwan.

GM1-gangliosidosis is an autosomal recessive lysosomal storage disease caused by a deficiency of acid beta-galactosidase (GLB1; EC3.2.1.23). Molecular analysis of the GLB1 gene has performed in two Taiwanese patients. The first patient (Patient 1) is a case of typical infantile, severe form, and the other one (Patient 2) had more severe presentations and a specific finding of cardiac involvement. Four novel mutations (H102D, A301V, G494V, c.945-7del) and one polymorphism (G23S) were identified. Patient 1 has found to have two missense mutations, H102D and A301V, and a G23S polymorphism. Patient 2 was presented with the G494V and c.945-7del mutations. Transient overexpression in COS-1 cells of cDNA encoding G494V, c.945-947del, A301V, H102D, G23S mutant b-galactosidases produced products with activities of 0, 0, 0, 12, 97.7 % compared with the cDNA clone for wild-type b-galactosidase. The mutations identified in patient 2 with cardiomyopathy are localized in the GLB1 gene region common to both the lysosomal b-galactosidase and b-galactosidase-like protein (also known as elastin binding protein, EBP), and may affect both molecules to present this specific clinical manifestations.

Association of Neurological Disability and X-Inactivation in Fibroblasts of Females Heterozygous for X-linked Adrenoleukodystrophy. *S.A. Fatemi, P.A. Watkins, A.B. Moser, L.M. Poetscher, N. Beauchamp, G.V. Raymond, H.W. Moser, S. Naidu.* Kennedy Krieger Institute, Baltimore, MD.

Objective: The degree of neurological involvement has been investigated extensively in affected males with X-linked adrenoleukodystrophy (X-ALD). However, there is limited data for heterozygote females. This study aimed to determine the range of neurological disability and its possible association with X-inactivation in fibroblasts of X-ALD heterozygotes. **Methods:** 76 X-ALD females between 8 and 76 years (median: 41.5, mean: 42.5 +/-15 years) were included in the study. The Kurtzke Expanded Disability Status Scale (EDSS) was used to rate the neurological impairment (range, 0:no abnormalities - 10:death). Brain MR Imaging (MRI) was performed in all subjects. In a subgroup of 40 patients indirect immunofluorescence analysis of cultured skin fibroblasts was performed. An antibody raised against the C-terminal 18 amino acids of the human peroxisomal ALD protein (ALDP) revealed a punctate, peroxisomal immunostaining pattern and average percentages of immunopositive and immunonegative cells were determined. **Results:** Nine patients were completely asymptomatic, while 67 demonstrated neurological abnormalities (Mean EDSS: 2.5, max. 8). A mixed population of normal and abnormal fibroblasts was demonstrated with a mean of 43 +/-17 % normal ALDP positive cells. Prominent bilateral cerebral white matter abnormalities were observed in the MRIs of two girls (13 & 14 years old) and of a 42 year old woman. These patients had absent or severely reduced ALDP expression (0, 1 and 7% immunopos. cells in the 14, 13 and 43 year old, respectively) suggesting skewed X inactivation. Patients age ($p=0.007$) and average percentage of immunoneg. cells ($p=0.05$) explained 35% of the variability of the EDSS using multiple regression analysis in patients without MRI abnormalities. **Conclusion:** MRI changes similar to cerebral childhood X-ALD in males were detected in heterozygote females who showed either absence or very low ALDP expression in fibroblasts. Furthermore, advanced age and reduced ALDP expression in fibroblasts were associated with a higher degree of neurological disability in X-ALD heterozygotes.

Mutations in exon 1 of *MLYCD* reveal a critical role for protein targeting and post-translational modification.

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Malonyl-CoA decarboxylase (*MLYCD*) deficiency (OMIM 248360) is an autosomal recessive disorder characterised by malonic aciduria, developmental delay, seizure disorder, hypoglycemia, and cardiomyopathy. *MLYCD* genomic sequencing in six unrelated patients revealed three non-conservative missense mutations, two small frame-shifting deletions and two single base insertions that together accounted for 11/12 pathogenic alleles. Fibroblast cell lines were available from 5 of these patients and two previously reported patients with homozygous *MLYCD* mutations. Antisera raised to a C-terminal peptide detected a previously unrecognised 66 kDa *MLYCD* isoform in control fibroblasts. This band was undetectable in 3/7 patients cell extracts. In 3/7 patients the was substantially reduced in intensity including one with a 25-base deletion that changes the ORF between two possible initiating AUG codons (M1 & M40) thus ablating the mitochondrial signal peptide of *MLYCD* ensuring only the peroxisomal form of the protein is translated. One cell-line show an overall increase in protein levels with the 66 kDa band and a prominent smeary band of 68-83 kDa. This mutation (p.M40T) ablates the second AUG and thus only allows translation of the intramitochondrial form of *MLYCD*. Immunocytochemical analysis confirmed the predicted intracellular mislocalisation and demonstrated a third category of mislocalisation due to a mutation (p.G3D). Taken together these data suggest that both peroxisomal and mitochondrial localisation of *MLYCD* are critical for its normal function and that a novel targeting signal may reside in a 4 amino acid conserved N-terminal motif.

Nitrous oxide and 5,10-methylenetetrahydrofolate reductase deficiency. *R.R. Selzer¹, D.S. Rosenblatt², R. Laxova³, K. Hogan¹.* 1) Department of Anesthesiology, University of Wisconsin, Madison, WI; 2) Department of Human Genetics, McGill University, Montreal; 3) Department of Medical Genetics, University of Wisconsin, Madison, WI.

Nitrous oxide irreversibly oxidizes the cobalt atom of vitamin B12, inhibiting activity of the cobalamin-dependent enzyme methionine synthase. Methionine synthase converts 5-methyltetrahydrofolate and homocysteine to tetrahydrofolate and methionine, the principal substrate for methylation in many biochemical reactions including assembly of the myelin sheath, neurotransmitter substitutions, and DNA synthesis in rapidly proliferating tissues. We report the neurologic deterioration and death of a child anesthetized twice with nitrous oxide before the diagnosis of 5,10-methylenetetrahydrofolate reductase (MTHFR) deficiency was established. MTHFR catalyzes the synthesis of 5-methyltetrahydrofolate. Sequence analysis of MTHFR RNA transcripts and genomic DNA from the patient and family members, together with direct assays of fibroblast MTHFR activity, reveal that the enzyme deficiency was caused by a novel MTHFR mutation (1755G→A, which changes a conserved methionine to an isoleucine), co-inherited with two common MTHFR polymorphisms (677C→T, 1298A→C) each independently associated with depressed enzyme function. We propose that an acquired lesion of methionine synthase superimposed on an inherited lesion in folate metabolism caused the patient's death, with the observed neuropathologic and biochemical changes closely tied to those predicted by impairment of folate and methionine pathways, including demyelination, neuronal atrophy, and depressed levels of CSF biogenic amines. Because two of the three contributing variants are homozygous in over 10% of the population, routine use of nitrous oxide merits closer scrutiny. Whether children with these and related common polymorphisms in MTR, MTRR and CBS are predisposed to subtle but significant decrements in neurological performance following nitrous exposure in childhood remains an unanswered question.

Genetic Variation in a Gene Critical for Glutathione Synthesis. *M.L. Freeman¹, A.S. Willis², S.R. Summar³, S.M. Williams⁴, E. Dawson⁵, F. Barr⁶, M.L. Summar³.* 1) Radiation Oncology, Vanderbilt University Medical Center, Nashville, TN; 2) Molecular Physiology and Biophysics, Vanderbilt University Medical Center, Nashville, TN; 3) Pediatrics/Genetics, Vanderbilt University Medical Center, Nashville, TN; 4) Meharry Medical College, Nashville, TN; 5) BioVentures, Inc., Murfreesboro, TN; 6) Pediatric Cardiology, Vanderbilt University Medical Center, Nashville, TN.

Glutamate cysteine ligase (GCL), also known as g-glutamylcysteine synthetase, is heterodimeric enzyme that synthesizes the first step in the *de novo* synthesis of glutathione. Glutathione plays a role in a number of cellular processes, including the scavenging of free radicals to neutralize oxidative stress. The pathophysiology of several diseases, including neurodegenerative disease and progression of HIV infection, is thought to involve glutathione and oxidative stress. Polymorphisms in GCL have the potential to affect glutathione synthesis and, therefore, diseases in which glutathione may be important. We undertook the systematic screening for polymorphisms through single strand conformation polymorphism analysis of the exons and intronic flanking sequences of GCLC, the gene that encodes the catalytic subunit of GCL. We identified 11 polymorphisms in GCLC - 1 previously reported triplet repeat in the 5' UTR, 1 synonymous, 1 nonsynonymous, 2 insertion polymorphisms in the 3' UTR, and 6 intronic polymorphisms. We also determined allele frequencies for each polymorphism in group of 163 individuals that fits the demographics of the middle Tennessee area. Five polymorphisms have alleles with frequencies less than 5%; the others had alleles with a frequencies of 9% or greater. The nonsynonymous polymorphism C1384T is one of the rare polymorphisms and has been found only in individuals of African descent. These polymorphisms may provide a tool for association studies of diseases in which glutathione may be important.

X-linked adrenoleukodystrophy: clinical and biochemical findings in Brazilian patients. *R. Giugliani^{1, 3}, M. Wajner¹, A. Barschak¹, C. Cecchin¹, D. Zandon¹, G. Ferreira¹, G. Maegawa¹, L. Jardim^{1, 2}, L. Goulart¹, L. Sirtori¹, M. Chiochetta¹, M. Deon¹, C. Vargas^{1, 4}.* 1) Medical Genetics Unit, Hosp de Clinicas, Porto Alegre, RS, Brazil; 2) Department of Internal Medicine, UFRGS, Porto Alegre, Brazil; 3) Department of Genetics, UFRGS, Porto Alegre, Brazil; 4) Department of Clinical Analysis, UFRGS, Porto Alegre, Brazil.

Adrenoleukodystrophy (X-ALD) is an X-linked recessively inherited peroxisomal disorder, phenotypically heterogenous, characterized by progressive white-matter demyelination of the central nervous system and adrenocortical insufficiency. We investigated 32 male X-ALD patients varying in age from 7 to 39, diagnosed from 304 suspected patients referred for investigation. Plasma levels of very long chain fatty acids (VLCFA) were measured at our laboratory using gas chromatography (GC). Twenty five cases of childhood X-ALD and seven cases of adrenomyeloneuropathy (AMN) were diagnosed. Leukodystrophy, paraparesis and learning difficulty were the most frequent symptoms, appearing in 25, 13 and 12 of the patients, respectively. Physician awareness of X-ALD seems inadequate to judge by age at diagnosis and lengthy interval between the start of symptoms and diagnosis. This is the first published series of Brazilian patients with X-ALD. We determined signs and symptoms relevant for diagnosis, as early identification seems important for treatment outcome. In addition, diagnosis identifies carriers, who could benefit from genetic counselling and prenatal diagnosis.

Genotyping Errors and Variance Components Linkage Analysis on the X chromosome. *T.A. Daigneault.*
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Identifying genotyping errors is crucial when considering large genotype data sets, since undetected genotype errors can greatly reduce evidence for linkage. The problem is especially important because the shift to single-nucleotide polymorphisms (SNPs) will lead to a greater proportion of hard to detect genotyping errors that do not produce Mendelian inconsistencies. To date, most authors have focused on the detection and treatment of genotyping errors for autosomal data. Here, we extend these methods to X chromosome analysis and evaluate their utility in variance components linkage analysis of X linked traits. We simulated 20 markers for both a 1-cM SNP map (2 equi-frequent alleles/marker) and a 5-cM microsatellite map (4 equi-frequent alleles/marker) in various nuclear family configurations with between 2 and 5 siblings, with and without parental information. In each pedigree, we changed the genotype of one individual per family. We checked for genotyping errors by finding Mendelian inconsistencies, and conducting a sensitivity analysis of the likelihood. As expected, checking for Mendelian inconsistencies alone leaves many errors undetected. For example, only 0-9% of SNP genotyping errors and 5-35% of microsatellite genotyping errors resulted in Mendelian inconsistencies for families with 3 siblings and no parents. For both maps, the overall detection rate exceeded 70% in families with 4 or more same sex siblings when maternal genotypes were not available. Paternal genotypes made little difference, but having the mother genotyped was as efficient as having both parents genotyped (97-98% errors detected for all size families). The overall detection rate was higher for the X chromosome than for the autosome. For example, in the X chromosome SNP map 48% of the errors were detected for only 2 genotyped female siblings, but only 17% of the errors were detected in the autosome map. In families with 3 siblings, having one genotype error per family can lead to a 30% loss of the LOD score in variance components analysis. In these simulations, our error checking strategy recovered 80% of the lost LOD score. Our results and software will be helpful for investigators interested in the mapping of X-linked traits.

Experimental spinal cord injury temporal profiling identifies cell cycle genes associated with neuronal damage: possible SNP candidates. *S. Di Giovanni*^{1,2}, *S. Knoblach*², *A.I. Faden*², *E.P. Hoffman*¹. 1) Children's National Medical Center, Washington DC; 2) Dep. of Neuroscience, Georgetown U, Washington DC.

Spinal cord injury is a major cause of disability, its clinical manifestation is highly variable depending upon the intensity and the site of the trauma, but variability seems to be present also between different individuals who sustained a similar level of injury. Much of the functional deficit results from delayed cellular consequences of injury repair mechanisms, and these are mediated by a large set of genes, strongly temporally regulated, mainly involved in neuronal fate or survival. To define the temporal series of gene expression changes following spinal cord injury and to identify possible SNP candidates, we employed spinal cord injury experimental model for an expression profiling study. Rats were subjected to a controlled impact injury at T8-T9 by weight drop and were sacrificed at four time points (30 min, 4h, 24h and 7 days), with 4 to 6 individual rat spinal cords expression profiled at each time point (total 84 profiles) using the U34A,B,C Affymetrix genechip containing about 27000 probe sets, including ESTs. Genes showing 40% or more present calls in 26 profiles by Affymetrix analyses were retained for further analysis, and p values (>0.05) and fold changes (2 fold threshold) correlated, with temporal and functional clustering. Specific RNAs were verified by QMF-RT-PCR using infrared primers, protein level quantified by western blot and localized by ICC. We found induction of DNA damage-inducible genes and genes favoring cell cycle progression at 4 and 24 hours after injury. Temporal profiling also allowed us to look for new candidate genes by nucleating ESTs that were temporally significantly co-regulated with genes with known function. Changes in mRNA expression were associated with changes in respective proteins as shown by western blots and ICC. Cell cycle and DNA damage related proteins were frequently localized in neurons showing signs of DNA damage and apoptotic features and are therefore likely to be important in modulating delayed cellular damage following spinal cord trauma. These genes may also represent good candidates for SNPs discovery in humans.

C-Reactive Protein is linked to regions on chromosome 10 and 5 in families with myocardial infarction. *U. Broeckel¹, C. Hengstenberg², K. Maresso¹, B. Mayer², S. Holmer², J. Erdmann², G. Schmitz³, L.J. Martin⁴, A.G. Comuzzie⁴, H.J. Jacob¹, H. Schunkert².* 1) Human and Molecular Genetics Center, Medical College of Wisconsin, Milwaukee, WI; 2) Clinic of Internal Medicine 2, Universitaet Regensburg, Germany; 3) Department of Clinical Chemistry, University of Regensburg, Germany; 4) Department of Genetics. Southwest Foundation of Biomedical Research. San Antonio, TX.

C-reactive protein (CRP) is a highly sensitive acute-phase marker of inflammation. Several studies have shown that CRP is strongly correlated with long term prognosis in patients with coronary heart disease (CHD) as well as healthy individuals, independent of traditional cardiovascular risk factors. While environmental components as well as drugs have been shown to influence CRP levels, recent reports have demonstrated a significant genetic component. In order to identify chromosomal regions linked to CRP, we performed a total genome scan in 513 families (1,406 individuals) ascertained for myocardial infarction.

A variance component approach was used to estimate heritability and to test for linkage with CRP. Initial analyses screened for general covariates including age, gender, body mass index (BMI), and LDL. Diabetes, CHD, systolic and diastolic blood pressure, cigarette smoking, and various medications as qualitative phenotypes. Heritability of CRP in a reduced model of just age and gender was 36%. In a model also incorporating diabetes, smoking and BMI, heritability was estimated at 31%. Qualitative covariates controlling for the effects of lipid-lowering drugs and aspirin treatment did not remain as significant covariates. In subsequent multipoint linkage analysis we identified two loci for CRP. A maximum LOD score of 3.02 was found at 141 cM on Chromosome 10 in addition to a loci at 151 cM on Chromosome 5 with a LOD of 2.23. These loci do not overlap with previously identified susceptibility loci for MI in this population.

In conclusion, this study represents the first total genome scan for CRP, demonstrating a significant genetic component, and identifying two distinct loci linked to CRP serum levels.

Genome-wide association study to identify genes related to myocardial infarction. *K. Ozaki¹, Y. Ohnishi¹, A. Iida¹, R. Yamada¹, T. Tsunoda¹, A. Sekine¹, H. Sato², H. Sato², M. Hori², Y. Nakamura^{1, 3}, T. Tanaka¹.* 1) SNP Research Center, The Institute of Physical and Chemical Research (RIKEN), Tokyo, Japan; 2) Department of Internal Medicine and Therapeutics, Osaka University Graduate School of Medicine, Osaka, Japan; 3) Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan.

To clarify genetic backgrounds in the pathogenesis of acute myocardial infarction (MI), we have launched a genome-wide association study in a Japanese population using 100,000 cSNPs. As a first step, we are now genotyping 94 MI patients and 658 general population by high-throughput multiplex PCR-Invader assay method. We temporarily chose p value of 0.01 to be threshold to select SNPs for further verification by expanding sample size. At present, we have screened 24,757 SNPs and 98% of them did not meet this threshold. When the rest of the SNP loci were re-examined with additional 1,000 MI patients and 1,000 general population, almost all of them did not show statistically significant association. However, one SNP locus showed a tight association, with a c^2 value of 21.6 ($p=0.0000033$) and odds ratio of 1.78 (95% confidence interval 1.39-2.27). We are now making a dense SNP map of this locus for linkage disequilibrium mapping, followed by functional analyses of gene products.

Examination of Candidate Chromosomal Regions for Type 2 Diabetes (T2DM) Reveals Evidence for a New Susceptibility Locus and Potential Epistatic Effects. *M.G. Pezzolesi¹, M. Nam¹, T. Nagase¹, T. Klupa¹, J.S. Dunn¹, S.S. Rich², J.H. Warram¹, A.S. Krolewski¹.* 1) Dept Genetics & Epidemiology, Joslin Diabetes Ctr, Boston, MA; 2) Wake Forest University School of Medicine, Winston-Salem, NC.

The search for major genes affecting the development of T2DM has been plagued by inconsistent linkage results. In a panel of 43 large Caucasian pedigrees in which diabetes segregates consistent with an autosomal dominant pattern, we genotyped markers in eight chromosomal regions previously reported as supporting linkage with T2DM. Previously, we have reported significant evidence of linkage on chromosome 20q (maximum LOD score, MLS=3.85) in this same panel. In the present analysis, candidate regions on 2q, 3q, 5q, 9q, and 10q yielded little evidence for linkage, regions on 1q (MLS=1.01) and 2p (MLS=0.67) gave only suggestive evidence of linkage, while a region on 8p (MLS=3.10) gave significant evidence of linkage. After increasing marker density on 8p, the MLS increased to 3.95 and the location shifted to a more telomeric position. In addition, conditional analyses were performed for each region and with our chromosome 20q data. The MLS for 1q and 20q significantly increased when conditioned on 2p (to 2.12 and 3.89, respectively) while the MLS at 8p decreased (from 3.95 to 1.75). When conditioned on 2p, the MLS for 9q increased to 1.15 (from 0.13), and the MLS for 9q increased to 0.52 (from 0.13) when conditioned on 20q. There did not appear to be an effect of conditioning on support for linkage to chromosome 8p.

In conclusion, while we were able to replicate suggestive linkage in our pedigrees on chromosomes 1q and 2p, we did not find evidence of linkage where previously reported on 2q, 3q, 5q, 9q, and 10q. However, our data did provide strong evidence to support a new susceptibility locus on chromosome 8p that appeared independent of other susceptibility loci in effect. Lastly, our conditional analysis supports evidence for two potential epistatic effects: interactions between 2p and (1q and 9q) and between 20q and (9q and 2p).

Otosclerosis and *COL1A1*. *W. Chen*¹, *M.J. McKenna*², *G. Van Camp*³, *R.J.H. Smith*¹. 1) Molecular Otolaryngology Research Laboratories, University of Iowa, Iowa City, IA, USA; 2) Department of Otolaryngology, Harvard Medical School, Boston, Massachusetts, USA; 3) Department of Medical Genetics, University of Antwerp, Antwerp, Belgium.

Statement of Purpose. Otosclerosis, a type of conductive deafness caused by abnormal bone homeostasis of the otic capsule, represents a frequent cause of hearing impairment among white adults. Although no causative genes have been cloned, a candidate gene genetic association study between otosclerosis and three collagen genes linked to mild forms of osteogenesis imperfecta (*COL1A1*, *COL1A2* and *COL2A1*) found a statistically significant association between otosclerosis and *COL1A1* (McKenna et al. 1998). We hypothesized that if this association is robust, it could be confirmed by single nucleotide polymorphism (SNP) genotyping. **Methods Used.** Proband from 130 families segregating otosclerosis were genotyped for six SNPs tightly linked to *COL1A1*. Data were compared to SNP genotypes of 174 controls. **Summary of Results.** One of the SNPs we genotyped is within a distinct cis element that regulates basal expression of *COL1A1* in osteoblasts. The SNP results from the deletion of one of eight consecutive thymidines. The genotypes of the 130 persons with otosclerosis were (TT, 52%; CT, 42%; CC, 6%); the genotypes of the controls were (TT, 73%; CT, 24%; CC, 3%). These differences are significant at the $p < 0.0006$ level (chi-square and exact test). **Conclusion.** This association suggests a common molecular basis of inheritance between some cases of otosclerosis and osteogenesis imperfecta. It also suggests that perturbations in the basal expression of *COL1A1* may be etiologically related to otosclerosis. Further investigation of this gene will be required to clarify its role in the pathogenesis of this disease. (Supported in part by NIH grant R01DC05218 to GVC and RJHS.).

A Refined Interval for the Autosomal Recessive Nonsyndromic Deafness Locus DFNB6. *C.A. Hodgkinson^{1,3}, S. Bohlega², S.N. Abu-Amero¹, M. Kambouris¹, E. Cupler², B.F. Meyer¹, V.A. Bharucha^{1,3}.* 1) Department of Genetics, KFSH&RC, Riyadh, Saudi Arabia; 2) Department of Neurosciences, KFSH&RC, Riyadh, Saudi Arabia; 3) Prince Salman Centre for Disability Research, Riyadh, Saudi Arabia.

Autosomal recessive nonsyndromic hearing loss has been linked to more than thirty different loci. The DFNB6 locus was previously identified in a single family and localized on chromosome 3p to a 14- to 20-cM interval bounded by the distal marker D3S1619 and the proximal marker D3S1766. We have identified a family exhibiting both autosomal recessive nonsyndromic deafness and autosomal recessive paraplegia. The paraplegic trait links to chromosome 13q14, but the deafness was excluded from this locus. In the course of performing a whole genome scan to localize the paraplegia locus we have been able to map the deafness to a 3Mb region on chromosome 3 that is completely contained within the interval previously described for DFNB6. It is therefore likely that DFNB6 and the gene at our locus are allelic. A recombination event in an affected individual localizes the underlying gene to a region bounded by D3S1298 and D3S3522. Additional mapping is currently being performed to further narrow the interval using microsatellite markers and Single Nucleotide Polymorphisms (SNPs). Also candidate genes for DFNB6 located within the interval are being interrogated by Mutation Detection Enhancement (MDE) Heteroduplex analyses and sequencing in an attempt to identify the causative gene.

Simultaneous phenotypic and genotypic dissection of neurodegeneration: Findings from the Kuopio Brain Bank.

*E.H. Corder*¹, *M. Pirskanen*², *M. Hiltunen*², *S. Helisalmi*², *A. Mannermaa*², *A. Koivisti*³, *H. Soininen*³, *M. Lehtovirta*³, *P. Hartikainen*³, *K. Ropponen*⁴, *R. Pirinen*⁴, *J. Bhm*⁴, *I. Alafuzoff*^{4,5}. 1) Demographic Studies, Duke Univ, Durham, NC; 2) Department of Clinical Genetics, Kuopio University Hospital; 3) Department of Neurology, Kuopio University Hospital; 4) Department of Pathology, Kuopio University Hospital; 5) Departments of Neuroscience and Neurology, Kuopio University.

Detailed clinical, autopsy and genetic information was investigated for the 850 subjects accessioned by the Kuopio Brain Bank. A pattern recognition approach called grade-of-membership analysis or GoM allowed identification of latent groups from numerous input variables. In this instance, six model-based groups were identified. They were defined by frequencies for the input variables and labeled as follows: 'No brain pathology' (I), 'Early death' (II), 'Cerebral infarcts' (III), 'Atherosclerosis & entorhinal AD' (IV), 'Limbic AD' (V), and 'Isocortical AD (VI)'. The order was established by increasing age at death. The labels summarize the pathologic findings. Importantly, genotypic frequencies for apolipoprotein E (APOE), alpha-2-macroglobulin (A2M) and interleukin-1-alpha (IL-1a) differed for the pathologic groups. The expected APOE associations were found: the frequency of e4/- and e4/4 increased with AD stage (e4+: 0% (IV), 51% (V), 73% (VI)) and was elevated for early death often due to MI or pulmonary embolism (46% (II)) and CNS infarct (44% (III)); e2/3 was protective. The highest A2M GG frequencies were found for limbic and isocortical AD (23%). Limbic AD was associated with AG or GG + APOE e34. IL-1a TT was associated with atherosclerosis not AD (21% (IV), 14% (V), 2% (VI)). These findings demonstrate that GoM may be a useful tool to relate sets of genotypes to multiple pathologic processes.

Empirical evaluation of pedigree stratification confirms parametric linkage to systemic lupus erythematosus (SLE) at 11q14 in African-American pedigrees with hemolytic anemia. *J.R. Kilpatrick¹, J.A. Jelly¹, J.B. Harley^{1,2,3}.*
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The parametric LOD score method testing specific models of inheritance provides evidence of linkage at 11q14 with SLE in African-American pedigrees stratified by the presence of hemolytic anemia in at least one SLE affected family member (LOD = 4.5). In this strategy, 16 pedigrees were selected with hemolytic anemia from 56 African-American pedigrees multiplex for SLE. Six models of inheritance were tested and the highest LOD score recorded. The LOD score obtained should be interpreted allowing for multiple testing across the models considered and the 308 microsatellite markers in the genome scan. We, therefore, simulated the assignment of genotypes to the founders, the meiosis on and inheritance of chromosomal segments in pedigrees, and the six screening models applied in 10,000 replicates of the 16 pedigrees. This produced a normal distribution at every locus. The observed data from the original genome scan was most significant at the locus allegedly linked with $p = 1.06e-07$, closely corresponding to the original LOD score obtained. The only other locus showing a possible confirmatory effect was found at 5p15 with $p = 0.008$ where LOD=2.1 was previously found. A new effect was also found at 11p11 with $p = 0.0004$, which is more significant than the LOD=1.8 found in the same location in the original genome scan. These results show for this example that testing multiple models of inheritance in parametric linkage analysis does not significantly inflate the likelihood of false positive linkage.

Linkage analysis of minor histocompatibility genes causing graft-versus-host disease (GVHD) following allogeneic bone marrow transplantation. *J. Ohashi*¹, *E. Maruya*², *K. Tokunaga*¹, *H. Saji*². 1) Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; 2) HLA Laboratory, NPO, Kyoto, Japan.

Acute graft-versus-host disease (GVHD) is a major cause of morbidity and mortality after bone marrow transplantation. Incompatibility of the major histocompatibility antigen (HLA) is a primary cause of GVHD. However, even if bone marrow donor and recipient have identical HLA, GVHD still occurs 10-35%, indicating that genes outside of the HLA region, generally called immuno-dominant minor histocompatibility antigens (mHas), may be responsible for the development of acute GVHD. In order to detect mHas or GVHD loci, Lunetta and Rogus [(1998) *Genet Epidemiol* 15:595-607] have recently proposed a linkage analysis for transplant sib pairs in which GVHD has developed in the recipient (GVHD+). In this study we developed a new linkage approach where sib pairs in which GVHD has not developed in the recipient (GVHD-) are used as well as GVHD+ pairs. This new approach is regarded as a combination of linkage analyses for affected sib pairs and for discordant (affected/unaffected) sib pairs. Thus, four parameters are maximized here to obtain the maximum likelihood. In order to test a null hypothesis (no linkage) against an alternative hypothesis (linkage), a likelihood ratio statistic is calculated, and a computer simulation is performed for the calculation of P-value. A computer simulation showed that the use of GVHD- pairs possibly increases a statistical power of linkage analysis. We also performed a linkage analysis for our reported data of several candidate variants of mHas [Maruya et al. (1998) *Blood* 92:2169-2176]. Our results suggested that a linkage analysis is powerful for detecting a GVHD gene even in a candidate gene approach.

Evidence that Smith-McCort dysplasia and Dyggve-Melchior-Clausen dysplasia are allelic disorders that result from mutations in a gene on the long arm of chromosome 18. *N. Ehtesham¹, R.M. Cantor^{2,3}, L.M. King¹, K. Reinker⁵, B.R. Powell⁶, A. Shanske⁷, S. Unger⁸, D.L. Rimion^{1,2,3,4}, D.H. Cohn^{1,2,4}.* 1) Medical Genetics, Cedars-Sinai Research Institute, Los Angeles, CA; 2) Depts of Human Genetics; 3) Medicine and; 4) Pediatrics, UCLA Sch. of Medicine, Los Angeles, CA; 5) Univ. of Texas Health Sciences Center, San Antonio, TX; 6) Children's Hospital Central California, Madera, CA; 7) Children's Hospital at Montefiore, Albert Einstein College of Medicine, Bronx, NY; 8) Div. of Clinical and Metabolic Genetics, Hospital for Sick Children, Toronto, Canada.

Smith-McCort dysplasia (SMC) is a rare autosomal recessive osteochondrodysplasia characterized by short limbs and a short-trunk with a barrel-shaped chest. The radiographic phenotype includes platyspondyly, generalized abnormalities of the epiphyses and metaphyses, and a distinctive lacey appearance of the iliac crest. Electron microscopy of chondrocytes demonstrates dilated cisternae of rough endoplasmic reticulum containing granular material. Radiographic and histological features of SMC are identical to Dyggve-Melchior-Clausen syndrome (DMC), a related autosomal recessive osteochondrodysplasia. However, patients with DMC also exhibit mental retardation. We performed a genome-wide scan in a consanguineous SMC family from Guam and found evidence for linkage to a region on chromosome 18q. Analysis of a second smaller SMC family was also consistent with linkage to this region, producing a maximum combined 2-point LOD score of 3.04 at a recombination fraction of zero for the marker at locus D18S450. A 10.7 cM region containing the disease gene was defined by recombination events in the larger family. Furthermore all affected children in that family were homozygous for a subset of marker loci within this region, defining a 1.5 cM interval likely to contain the defective gene. Analysis of three small, unrelated families with DMC provided evidence of linkage to the same region, consistent with the long-standing hypothesis that the two disorders are allelic. Thus the eventual identification of the disease gene is expected to define a new gene important for both skeletal development and brain function.

Genome Scan for QTLs Underlying Bone Size Variation at Ten Refined Skeletal Sites: Genetic Heterogeneity and the Value of the Subdivision of Traits. *Q.Y. Huang, F.H. Xu, H. Shen, H.Y. Deng, T. Conway, Y.J. Liu, Y.Z. Liu, J.L. Li, K.M. Davis, R.R. Recker, H.W. Deng.* Biomed Sci/Osteoporosis Res Ctr, Creighton Univ, Omaha, NE.

Bone size is an important determinant of osteoporotic fractures. To identify QTLs underlying variation in bone size, we conducted a whole-genome linkage scan in 53 pedigrees ascertained through a proband having BMD Z-scores < -1.28 at the hip or spine. Three hundred eighty microsatellite markers with an average spacing of 8.6 cM were genotyped. Lumbar 1 area, lumbar 2 area, lumbar 3 area, lumbar 4 area at the spine, femoral neck area, trochanter area, intertrochanter area at the hip, ultradistal radius area, mid-distal area and one-third distal area at the wrist, were measured by dual energy X-ray absorptiometry (DXA), and adjusted for age, height, weight, and sex. Two-point and multi-point linkage analyses were performed for each skeletal bone size, their composite measures and principal components using variance-components method implemented in the SOLAR package. Three chromosomal regions were identified with significant evidence of linkage (LOD>3.3) to bone size in at least one skeletal site: 1q22 and 10q21, for one-third distal area; 11p15, for lumbar 3 area; and fifteen with suggestive evidence (LOD>1.9), 2p25 and 1p13-21, for lumbar 1 area; 4q22-23, for lumbar 2 area; 7p21, for lumbar 3 area; 20p13, for lumbar 4 area; 2p24 and 7p14-15, for femoral neck area; 5p15 for trochanter area; 14p11 and 19p11-13, for intertrochanter area; 17q23, 2q37 and 9q21, for ultradistal radius area; 11q13-14, 14p11, 18q21, and 20q11-13, for one-third distal area. Our results indicated that the low power of QTLs mapping for principal components and composite measurements may result from genetic heterogeneity of complex traits. Results point to the value of the subdivision of traits to improve the power for detecting linkage.

Autosomal dominant familial calcium pyrophosphate dihydrate deposition disease (CPPDD) is caused by mutation in the transmembrane protein ANKH. *C.J. Williams¹, Y. Zhang², A. Timms², G. Bonavita¹, F. Caeiro³, J. Broxholme², R. Marchegiani³, A. Reginato⁴, R.G.G. Russell⁵, B.P. Wordsworth², A.J. Carr⁶, M.A. Brown^{2,5,6}.* 1) Dept Medicine/Rheumatology, Thomas Jefferson Univ, Philadelphia, PA; 2) Wellcome Trust Centre for Human Genetics, Oxford, U.K; 3) Hospital Privado, Cordoba, Argentina; 4) Division of Rheumatology, University of Medicine and Dentistry of New Jersey, Camden, NJ; 5) Oxford University Institute of Musculoskeletal Research, Oxford, U.K; 6) Nuffield Department of Orthopaedic Surgery, Nuffield Orthopaedic Centre, Oxford, U.K.

Familial autosomal dominant calcium pyrophosphate dihydrate (CPPD) chondrocalcinosis has previously been mapped to chromosome 5p15 in British, French and Argentinian pedigrees (CCAL2 - OMIM 118600). The ANKH gene which encodes a putative inorganic pyrophosphate transport transmembrane protein is encoded in this region. We sequenced all 12 exons and their exon-intron boundaries and 500bp of the promoter region in an Argentinian pedigree with autosomal dominant CPPD chondrocalcinosis. In all, 6 SNPs were identified and 2 insertions in the promoter region. One variant, a C to T transition at 14bp from the start codon, segregated with the disease in the family. Linkage of the mutation in the family was demonstrated with a LOD score of 9.6. This sequence change is predicted to cause a proline to leucine substitution at amino acid position 5. We did not observe this variant in 50 Northern Italian, 52 South American Hispanic, or 100 healthy British Caucasian controls, nor in 100 non-familial British Caucasians with CPPD chondrocalcinosis. Structural prediction programs were used to investigate the ANKH protein. Whilst these confirmed that the ANKH protein is almost certainly a transmembrane protein, the number of predicted transmembrane domains varied between 7 and 12, and agreement between programs as to the predicted orientation and structure was not high. We predict that as loss of function mutations of ANKH cause excess calcium hydroxyapatite deposition, the mutation that we have identified causes gain of ANKH function, raised extracellular inorganic pyrophosphate levels, and hence CPPD chondrocalcinosis.

Confirmation of the mapping of an Intracranial Aneurysm Predisposition Locus to Chr 7. *L.A. Cannon-Albright¹, J. Farnham¹, N.J. Camp¹, S. Neuhausen¹, G. Wood¹, G. O'Neil¹, H. Buswell², J. Tsuruda², D. Parker², J. MacDonald³.*
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Onda et al (2001) recently reported a significant linkage to chromosomal region 7q11 in a genomic search using 85 Japanese nuclear families with at least 2 affected sibs (104 sib pairs). In an attempt to replicate this finding, we performed linkage analysis in 12 extended high-risk IA pedigrees from Utah, comprising 44 IA cases. These pedigrees were ascertained from both a Utah genealogy resource linked to Utah death certificates (representing clusters of 2 or more individuals with a cause of death of aneurysm) or were self-ascertained in response to notices of the research study. All first-degree relatives of IA cases were screened with high-resolution MRA to detect aneurysms. Previously diagnosed IAs were confirmed by review of medical records and images. We genotyped 3 markers in the region of the maximum LOD reported by Onda et al (2001), covering a range of approximately 2cM. We performed two-point and multipoint parametric linkage analysis, employing general dominant and recessive models (moderate penetrance and very low sporadic rate). We used 2 diagnostic schemes (confirmed cases only, and all cases--which included 12 IA cases reported by family members, but not yet confirmed). Analyses utilizing the recessive model and including all cases yielded significant confirmation (two-point LOD = 2.18, theta = 0.0, p = 0.002 (D7S2518); multi-point LOD = 1.91, near D7S2518, p = 0.003. Our study is the first to confirm the linkage of the 7p11 locus in IA. This chromosome 7 region contains the elastin gene (ELN). Onda et al (2001) also reported a strong association with IA and a haplotype between the intron-20/intron-23 polymorphism of ELN (p=3.8 X 10⁻⁶). Analysis of additional markers and additional pedigrees, as well as association studies of SNPs in ELN will clarify whether ELN is the IA predisposition gene localized.

Candidate region on chromosome 20 for very-late onset Alzheimer Disease. *K.A.B. Goddard¹, J.M. Olson¹, H. Payami², M. van der Voet³, H. Kuivaniemi³, G. Tromp³.* 1) Epid/Biostat, MetroHlth Med Ctr, Case Western Reserve Univ, Cleveland, OH; 2) Neurology, Oregon Health Sciences Univ, Portland, OR; 3) Ctr Mol Med & Genetics, Wayne State Univ, Detroit, MI.

Recently, we reported a novel candidate region on chromosome 20 using a covariate-based affected sib-pair (ASP) linkage method to analyze 272 ASPs recruited as part of the NIMH Alzheimer Disease (AD) Genetics Initiative (Olson et al., *Am J Hum Genet*, 2002). ASPs linked to this region appear to be those with the oldest current age and, to a lesser extent, those with ApoE2 alleles. The 15 cM candidate region between D20S186 and D20S200 contains one particularly interesting candidate gene, cystatin C (CST3), which is located 1 cM from D20S200. CST3 is associated with late-onset AD, and co-deposits with amyloid- β in the brains of AD patients. We initiated a case-control study to further evaluate this candidate region. The cases include 49 NIMH ASPs (89 subjects) in the top 15% for mean current age, who are most likely linked to the candidate region. The controls are 129 healthy subjects recruited as part of a longitudinal study at OHSU, who have undergone extensive neuropsychological testing, and who met strict criteria for lack of dementia at study entry. The groups have comparable ages of 82-105 yrs for the cases (mean of 92 yrs), and 86-103 yrs for the controls (mean of 91 yrs). We examined microsatellite markers in the region and a CST3 haplotype of three SNPs in complete disequilibrium, including one that encodes an Ala/Thr polymorphism, for evidence of association with AD. The strongest evidence of association was found with D20S200 (c2 test; $p < 0.0001$). In addition, we constructed haplotypes for the 49 NIMH ASPs and their unaffected siblings, and identified a group of related haplotypes that are shared identical-by-descent (IBD) by ASPs in 61% of the families. Thus, we have identified a genetically homogeneous subset of very-late onset AD ASPs that share a group of related haplotypes IBD on chromosome 20, providing strong evidence of a susceptibility locus in this region. We continue to genotype additional markers to narrow the candidate region, and to further evaluate CST3 as a candidate locus.

Clinical and genetic studies in hereditary spastic paraplegia with thin corpus callosum. *C. Casali¹, E.M. Valente², E. Bertini³, G. Montagna³, C. Criscuolo⁴, G. De Michele⁴, M. Damiano¹, A. Pierallini⁶, F. Brancati², V. Sarano⁴, A. Rossi⁵, A. Tessa³, F. Cricchi¹, M. Muglia⁷, M. Carella⁸, G.A. Amabile¹, G. Nappi⁹, A. Filla⁴, B. Dallapiccola², F.M. Santorelli^{1,3}.* 1) Dept. Neurology, ORL & Rehab, La Sapienza Univ, Rome, Italy; 2) CSS-Mendel Institute, Rome; 3) Molecular Medicine and Department of Neurosciences, IRCCS-Bambino Ges, Rome; 4) Department of Neurological Sciences, Federico II University, Naples; 5) Institute of Neurological Sciences, University of Siena; 6) Department of Neurological Sciences, La Sapienza University, Rome; 7) Institute of Experimental Medicine and Biotechnology, CNR, Mangone (CS); 8) Telethon Institute of Genetics and Medicine (TIGEM), Naples; 9) Molecular Neurobiology, IRCCS-C. Mondino, Pavia.

Hereditary spastic paraplegias (HSP) are a group of neurodegenerative disorders characterized by progressive and prominent spasticity of the lower limbs. Autosomal dominant, autosomal recessive, and X-linked forms have been described. A complicated form of recessive HSP with thin corpus callosum (TCC) was first described in Japan, and most Japanese families studied showed linkage to chromosome 15q13-15. A recessive HSP locus (SPG11) has been mapped to chromosome 15q13-15 in Italian and North American families with and without TCC; it overlaps with the region identified in the Japanese families. We investigated 18 affected and 30 healthy individuals from 12 unrelated Italian families with recessive HSP-TCC. Clinical, neurophysiological, and neuroradiological studies were undertaken. Linkage to 15q13-15 could neither be confirmed nor excluded with certainty in six families. Five families showed evidence for linkage, identifying a 19.9 cM region between markers D15S1007 and D15S978 encompassing the SPG11 interval. In one family, linkage could be firmly excluded, confirming genetic heterogeneity. Our findings suggest that HSP-TCC is more frequent in Italy than was previously believed. The phenotype consists of pyramidal tract involvement confined to lower limbs with subsequent diffusion to upper limbs, and progressive cognitive impairment. While a major locus for HSP-TCC exists on chromosome 15q13-15, there is evidence for at least one other locus.

Refinement of PARK7 critical region on chromosome 1p36. *V. Bonifati^{1,2}, O. Schaap¹, J.M van Baren¹, J. van Dongen¹, P. Rizzu¹, C.M van Duijn³, G. Meco², B.A. Oostra¹, P. Heutink¹.* 1) Clinical Genetics, Erasmus University, Rotterdam, The Netherlands; 2) Department of Neurological Sciences, La Sapienza University, Rome, Italy; 3) Department of Epidemiology and Biostatistics, Erasmus University, Rotterdam, The Netherlands.

Three loci (PARK2, PARK6, and PARK7) have been identified for autosomal recessive, early-onset parkinsonism (AREP). We mapped the PARK7 locus in a large Dutch consanguineous family and confirmed these findings in an Italian consanguineous family. Initial haplotype analyses delineated in both these PARK7-linked families a critical region of about 16 cM flanked by marker D1S243 (telomeric) and D1S244 (centromeric). In the current physical maps of the human genome the PARK7 region spans about nine megabases, including many gaps between different contigs. The order of the contigs is still provisional and the internal assembly for some contigs has low reliability. The PARK7 region was saturated by typing all microsatellite markers available. For some contigs we also genotyped SNPs present in the public and Celera databases. Moreover, we performed extensive recombination analyses using new, polymorphic short tandem repeats that we identified by scanning the genomic sequence using software developed in our bioinformatic core. We identified several non-consanguineous families consistent with linkage to the PARK7 region, however, due to the small family size and locus heterogeneity in AREP, we consider these additional families as possibly linked to PARK7 locus. Therefore, in the fine-mapping phase we only used the two PARK7-definitely linked families. A single-contig-based work strategy has allowed us to reduce the critical region to 5.5 megabases flanked by markers Rep26 and Rep33X, excluding a large number of candidate genes. The refined PARK7 minimal critical region contains around 90 genes and EST clusters, including many predicted or confirmed genes coding for proteins of unknown function. This work has considerably reduced the PARK7 critical region. Cloning the defective gene at the PARK7 locus will allow to evaluate its frequency of involvement in AREP, and to accurately define the associated phenotype.

Microsatellite marker-based genome-wide association studies for Parkinson's disease by using the pooled DNA method. *Y. Momose^{1, 2}, M. Murata², G. Tamiya³, T. Ikuta³, A. Oka³, K. Okamoto³, K. Kobayashi¹, M. Tachikawa¹, Y. Yoshikawa³, M. Yamamoto⁴, N. Hattori⁵, Y. Mizuno⁵, I. Kanazawa⁶, H. Inoko³, T. Toda¹.* 1) Div Functional Genomics, Dept Post-Genomics and Diseases, Osaka Univ Grad Sch Med, Osaka, Japan; 2) Dept Neurology, Grad Sch Med, Univ Tokyo, Tokyo, Japan; 3) Dept Molecular Life Science II, Sch Med, Tokai Univ, Kanagawa, Japan; 4) Dept Neurology, Kanagawa Pref Central Hosp, Kagawa, Japan; 5) Dept Neurology, Sch Med, Juntendo Univ, Tokyo, Japan; 6) National Inst Neuroscience, NCNP, Tokyo, Japan.

Parkinson's disease (PD) is a complex disorder, with multiple genetic and environmental factors influencing disease risk. Although several causal genes for Mendelian-inherited PD have recently been identified, certain genetic factors associated with pathogenesis of idiopathic PD have not yet been clarified. To identify susceptibility genes for PD, we started microsatellite marker-based genome-wide association studies by using the pooled DNA method. For initial screening, we made a pooled DNA each from 124 patients with PD and 124 normal controls and started association studies with 30,000 polymorphic microsatellite markers that are arranged at intervals of approximately 100kb. We analyzed PCR products with the GeneScan™ software and compared the pattern of PCR products of pooled DNAs from patients with PD and controls by the software PickPeak exploited by Ishibashi et al. We have finished to check approximately 1600 markers and found some significant associations on chromosome 1 where other studies showed a linkage. Genes in linkage disequilibrium with these markers may be associated with pathogenesis of PD. We will continue the genome-wide association studies to find the susceptibility genes for PD.

Autosomal recessive cerebellar agenesis maps to chromosome 22q11 in an Israeli Arab family. *L. Levin, N. Magal, L. Basel-Vanagaite, G. Halpern, M. Shohat.* Dept Medical Genetics, Rabin Medical Ctr, Petah Tikva, Israel.

Cerebellar agenesis, the absence of or only partial development of the cerebellum, can occur alone or as part of several syndromes including Joubert syndrome, which has been mapped in some families to 9q34.3, Lissencephaly (7q22), Meckel Syndrome (17q22-23), Rutledge Lethal Multiple Congenital Anomaly (11q12-q13), Hydroletharus Syndrome (11q23-q25), Spastic Ataxia, Charlevoix-Saguenay Type (13q12), Isolated Non-Ketotic Hyperglycinemia Type 1 (9p22), and Acrocallosal Syndrome (13p13.3-p11.2). These are all autosomal recessive disorders, with symptomatology similar to that of non-syndromic cerebellar agenesis. The symptoms and signs common to all include hypotonia, ataxia, nystagmus and developmental retardation.

We have recently seen a consanguineous family where the parents are first cousins and have six children. Three are normal, and three, all girls, have cerebellar agenesis with a typical clinical picture with no specific additional findings. We performed a linkage study in order to map the disease-causing gene in our family. We excluded linkage to each of the genes for the above-mentioned syndromes associated with cerebellar agenesis. We then performed a whole genome screening, which revealed linkage to D22S315 (chromosome 22q11) (max $Z = 2.7$, $q = 0.01$). The size of the original candidate locus was approximately 22Mb. We then carried out further linkage studies using polymorphic markers, and were able to reduce the size of the locus to approximately 5Mb. Mapping the gene in this family will provide a tool for prenatal diagnosis and will enable us to identify carriers within the small village where the family lives and where there are several other similarly consanguineous families. The discovery of the gene will further enhance our understanding of brain development.

Linkage analysis in an Italian family with autosomal recessive Hereditary Spastic Paraplegia. A. Magariello¹, A.L. Gabriele¹, R. Mazzei¹, F.L. Conforti¹, A. Patitucci¹, T. Sprovieri¹, L. Mangone², L. Morgante³, A. Epifanio³, P. La Spina³, M. Muglia¹. 1) Institutes of Neurological Sciences, National Research Council, Mangone, CS, Italy; 2) Institute of Neurology, School of Medicine, Catanzaro, Italy; 3) Neurology Clinic, University of Messina, Messina, Italy.

Hereditary spastic paraplegia (HSP) is a heterogeneous group of disorders of the motor system, characterized clinically by slowly progressive lower extremity spasticity and weakness, in which dominant, recessive and X-linked forms have been described. While autosomal dominant HSP has been extensively studied, autosomal recessive HSP is less well known and it is considered a rare form. Until now, five families (four Tunisian and one Algerian) with recessive HSP linked to chromosome 8p11-8q13 have been published. The HSP locus was mapped in a region of 32.2 cM flanked by the markers PLAT and D8S279. In the current study, we report a small Italian (AR)HSP family linked to chromosome 8. Using additional markers located between PLAT and D8S279, we were able to refine the HSP region. Negative lod scores were obtained in the two-point linkage analysis by using STR markers on chromosome 15 and 16, whereas positive lod scores were obtained for D8S509, D8S1828, D8S285, D8S1102, D8S1723, D8S260, D8S1840 and D8S279 with a maximum lod score of 1.46 at D8S260 marker. The two affected siblings were homozygous for the markers D8S1102, D8S1723 and D8S260. According to the Genethon map, haplotype reconstruction revealed that the patients shared a common 8.2 cM region of homozygosity encompassing the D8S1102, D8S1723 and D8S260 markers. Based on recombination events in our Italian family, we could map the (AR)HSP gene between D8S285 and D8S1840, thus refining the HSP region by approximately 20 cM, from 32.2 to 12cM.

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Genome-wide scan in 115 Attention Deficit Hyperactivity Disorder (ADHD) Affected Sibling Pair families. *M.N. Ogdie¹, I.L. Macphie², S.E. Fisher², C. Franks², S. Minassian¹, J.J. McCracken⁴, J.T. McGough³, A.P. Monaco², S.F. Nelson¹, S. Smalley³.* 1) Human Genetics, UCLA, Los Angeles, CA; 2) Wellcome Trust Centre for Human Genetics, Oxford University, Oxford, UK; 3) Dept. of Psychiatry, UCLA, Los Angeles, CA; 4) UCLA Neuropsychiatric Institute, Los Angeles, CA.

Attention Deficit Hyperactivity Disorder (ADHD) is a common, highly heritable neurobehavioral disorder with childhood-onset characterized by hyperactivity, impulsivity, and inattention. As part of an ongoing study into the genetic etiology of ADHD, we performed a genome scan in 115 nuclear families comprised of 479 individuals and 166 Affected Sibling Pairs (ASPs). This cohort constitutes a replication set for which the previous results have been reported (S.E. Fisher et al., 2002). Individuals were genotyped with a set of polymorphic microsatellite markers that define a ~10cM map across the entire genome. The current linkage analysis attempts to replicate the initial evidence for linkage, including multipoint LOD scores exceeding 1.5 for regions on 5p12, 10q26, 12q23 and 16p13, as well as identify additional candidate regions. We are conducting multipoint nonparametric analyses in MERLIN v 1.0.

Confirmation and refinement of the SPG5A locus for pure autosomal recessive hereditary spastic paraplegia. S.

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The term hereditary spastic paraparesis (HSP) is used to describe a group of clinically and genetically heterogeneous neurodegenerative disorders in which the predominant feature is progressive spasticity of the lower limbs. The phenotype is generally categorised as 'pure' when symptoms and signs are confined to those of a spastic paraparesis, or 'complicated' if there are additional neurological or other clinical features. The SPG5A locus, a 32cM region spanning the centromeric area of chromosome 8, was the first pure autosomal recessive form of HSP in which linkage was established using a small group of Tunisian families. Since then there have been no further reports of conclusive linkage to this locus. We have identified a large consanguineous English family with a similar pure HSP phenotype. Homozygosity mapping using microsatellite markers from chromosome 8 confirmed linkage to the SPG5A locus with a maximum multi-point LOD score of 4.84 between markers D8S1833 and D8S285. This has established that this form of HSP is not confined to the Tunisian population and points towards a possible common phenotype for SPG5A linked HSP. Loss of homozygosity occurred at markers D8S1051 and D8S1807 defining these as the flanking markers and refining the SPG5A locus to a 23cM region on the long arm of chromosome 8. Direct sequencing of the putative LOC157677 gene in affected individuals, whose predicted protein product is an AAA protein with a high degree of homology to the proteasome 26S subunit, has excluded this as a candidate gene from a causative role in the pathogenesis of this disease.

Association between SNPs at the PARK3 locus and Onset Age of Parkinson Disease. *S. Karamohamed, A. Herbert, A.L. DeStefano, C.M. Shoemaker, J.B. Wilk, K.M. Sullivan, G. Xu, J.S. Fink, R.H. Myers and GenePD study.* Neurology, Boston Univ Sch Medicine, Boston, MA.

Parkinsons disease (PD) is a common neurodegenerative disorder characterized by pathological degeneration of the substantia nigra, loss of dopamineergic neurons, and a symptom triad of rigidity, resting tremor and bradykinesia. Although evidence for potential susceptibility loci for PD on several chromosomes has been obtained by genome-wide scans no gene contributing to idiopathic PD has been mapped. In this study, a region on chromosome 2p14-p12 showing linkage to age at onset of PD, which harbors several candidate genes, was fine mapped using microsatellite and single nucleotide polymorphisms (SNPs) that were available from the NCBI database. MALDI TOF mass spectroscopy was used to evaluate SNPs either within or very near candidate genes spanning about 2 Mb. A total of 867 patients with PD, including more than 250 PD affected sibling pairs, and 249 controls were examined in this study. Association between SNPs and onset age of PD was assessed using regression analysis. Several SNPs close to potential candidate genes produced statistically significant association, including one (G/T, $P=0.006$) associated with about 8 years difference in age-onset. In addition, another SNP (G/A, $P=0.04$) located approximately 110 kb downstream of the region was associated with an approximately 7 years difference in age-onset between PD patients who do and do not possess the rare allele. This region is being further evaluated using additional SNPs in order to fine map the area.

Hereditary sensory neuropathy with cough and gastroesophageal reflux linked to chromosome 3. *C. Kok¹, M.L. Kennerson^{1,2}, P.J. Spring³, A.J. Ing⁴, J.D. Pollard³, G.A. Nicholson^{1,2}.* 1) Neurobiology, ANZAC Research Institute, Sydney, NSW, Australia; 2) Molecular Medicine Laboratory, Concord Hospital, Concord, NSW, Australia; 3) Institute of Clinical Neurosciences, Royal Prince Alfred Hospital & University of Sydney, NSW, Australia; 4) Department of Respiratory Medicine, Concord Hospital, NSW, Australia.

Hereditary sensory neuropathy type 1 is a group of dominantly inherited degenerative disorders of the peripheral nerves. A new form of HSN 1, HSN with gastroesophageal reflux (GOR) and cough has only recently been recognised by Pollard and colleagues. Initial clinical and linkage studies excluding known hereditary neuropathy and GOR loci have been described by Spring et al and Nicholson et al (in press, *Muscle and Nerve*, Abstracts of Xth Congress on Neuromuscular Diseases). The onset of the cough is between the second and the fourth decades with repeated throat clearing, often induced by lying flat. Later patients can suffer from paroxysms of cough, which occurs spontaneously, after eating, or after inhalation of strong odours and/or perfumes. 24 Hour oesophageal pH monitoring, shows that the cough is temporally related to acid reflux in the distal and proximal oesophagus. Sensory loss, mainly affecting the feet and legs, occurs later. Neurophysiological studies show an axonal neuropathy. To map the chromosomal location of the gene mutation causing the disorder, 26 members of a large family were recruited including 4 affected females and 4 affected males. A genome wide screen using 383 microsatellite markers at 10 cM intervals was undertaken. Two point linkage analysis has shown the most likely location for the gene causing HSN with cough and GOR lies on chromosome 3 ($Z_{max}= 4.17$ at $\theta=0.0$). Further studies are continuing to refine the genetic interval and to carry out positional cloning and candidate gene screening.

A GABRA6 Coding Variant is associated with the NEO-PI Domain Neuroticism, a Risk Factor for Depression.
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Psychiatric disorders are considered to be influenced by multiple genes and the environment. An increased interest has arisen in the study of personality traits as risk factors that are correlated with depression and/or severity of depression. The strong genetic basis of such traits increases the power to detect genes that confer vulnerability to depression. As has been proposed, high scorers of Neuroticism, measured by the NEO Personality Inventory (NEO-PI), have shown a significant and consistent association with depression. The Gamma-aminobutyric acid (GABA) system has been extensively studied in psychiatric disorders such as alcoholism, anxiety and most recently in unipolar depression where cortical GABA levels are reduced. Furthermore, evidence for linkage to bipolar disorder has been reported by several independent studies to chromosome 5q. These lines of evidence prompted us to investigate a coding SNP, Pro385Ser, located in the second intracellular domain of the GABA A6 receptor (GABRA6) subunit adjacent to a putative phosphorylation site, in 391 subjects of a large community sample (Tecumseh sample) who completed the NEO-PI questionnaire. The more common, Pro-Pro genotype was associated with significantly higher mean Neuroticism scores ($p = 0.007$). Within the Neuroticism domain, the facets Anxiety, Depression, Hostility, and Vulnerability were associated with GABRA6 Pro-Pro genotype ($p = 0.026$, $p = 0.014$, $p = 0.011$ and $p = 0.013$ respectively). Our results suggest that GABRA6 should be investigated as a candidate gene for depression and bipolar disorder.

A strong association of the *XmnI* promoter polymorphism of the gamma globin gene (HBG2) to Alzheimers Disease in the NIMH sibling dataset. Perry, R.T., Hill, W.D., Wiener, H., Harrell, L., Gearhart, D.A., Kutlar, F., Kutlar, A., Blacker D., Tanzi R.E., McInnis M.G., Bassett S.S., Go, R.C.P. R.T. Perry¹, W.D. Hill^{2,3}, H. Wiener¹, L. Harrell¹, D.A. Gearhart^{2,3}, F. Kutlar², A. Kutlar², D. Blacker⁴, R.E. Tanzi⁴, M.G. McInnis⁵, S.S. Bassett⁵, R.C.P. Go¹.
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Proteins that interact with b-amyloid peptides (Ab) may mediate Ab toxicity or deposition and thus contribute to the pathogenesis of Alzheimer's disease (AD). While using a phage display of a human brain cDNA library to identify proteins that interact with Ab, the gamma (G)-globin subunit of fetal hemoglobin (Hb F) was identified and shown to bind to Ab, an interaction later confirmed by surface plasmon resonance. Later, we determined Ab 1-42 permissively binds to the oxidized metHb forms of both adult and Hb F but not to the normal reduced forms, and that adult metHb binds with significantly higher affinity than the fetal form. Free Hb, released from damaged RBCs, is normally oxidized in the plasma to metHb. We hypothesize that this metHb can interact with free plasma Ab and release the heme group. Free heme readily enters endothelial cells and is a potent source of reactive oxygen species that can cause damage to the cells - similar to AD vascularpathology. We used family-based association testing to look at the *XmnI* polymorphism (C®T) at bp -158 in the promoter region of the G-globin gene (HBG2) in the NIMH sibling dataset (n=481 familes). In the presence of various stressors this *XmnI* SNP causes an increase in Hb F expression in adults. We found a significant protective association of this SNP to AD in families with at least two affected and one unaffected siblings (p=0.005), with an age of onset > 50 years (p=0.010) and > 70 years (p=0.013), and families not homozygous for the APOE4 allele (p=0.015). Hb F may be less toxic than adult Hb in its interaction with Ab and a higher level of G-globin expression may be relatively protective when there is a high level of vascular Ab.

Linkage mapping of a novel susceptibility locus for moyamoya disease to chromosome 8q22. *K. Sakurai¹, J. Nakayama¹, H. Ikeda², T. Yoshimoto², K. Ikezaki³, M. Fukut³, T. Arinami¹.* 1) Medical Genetics, Basic Medical Sciences, Tsukuba, Ibaraki, Japan; 2) Department of Neurosurgery, Tohoku University Graduate School of Medicine, Sendai, Iwate, Japan; 3) Department of Neurosurgery, Tohoku University Graduate School of Medicine, Kyushu University, Fukuoka, Fukuoka, Japan.

Moyamoya disease (MIM 252350) is characterized by stenosis or occlusion of the terminal portions of the bilateral internal carotid arteries and by abnormal vascular networks at the base of the brain. There is a high incidence of moyamoya disease in Asia, especially in Japan. Although most of the cases appear to be sporadic, within 10% occur as familial cases. Multifactorial inheritance is estimated with lambda s greater than 40. Thus the genetic factor for moyamoya disease has been expected and there have been several linkage studies of families with moyamoya disease, and indicated that susceptibility loci for the disease are located on chromosomes 3p, 6q, and 17q. We searched for loci linked to the disease by a genome-wide screening on 12 nuclear families with moyamoya disease-affected sib-pairs (46 members, 12 sib-pairs, 2 families lacking a paternal sample), using 428 microsatellite markers. We used the nonparametric linkage (NPL) score (Zall) and MLS for multipoint analysis and Zlr and nonparametric LOD statistics for single point analysis. Transmission disequilibrium test (TDT) was done with the SIB-PAIR program. We found significant evidence for linkage to 8q22 [maximum LOD score (MLS) of 3.6] and suggestive evidence for linkage to 12p13 (MLS = 2.3). The present study provides a novel locus for moyamoya diseases.

Catatonic schizophrenia: confirmation of linkage to chromosome 15 and further evidence for genetic

heterogeneity. A. Reis^{1,2}, F. Ruschendorf^{2,3}, D. Seelow^{2,4}, A.B. Ekici^{1,2}, G. Stober⁵. 1) Inst. of Human Genetics, University of Erlangen-Nuremberg, 91054 Erlangen, Germany; 2) Gene Mapping Center, Max-Delbruck-Center, Berlin; 3) Inst. of Medical Biometry, Informatics and Epidemiology, University of Bonn; 4) Fachbereich Biologie, Chemie, Pharmazie, Free University Berlin; 5) Dept. of Psychiatry and Psychotherapy, University of Wurzburg.

We earlier reported on significant evidence for linkage on chromosome 15q15 in periodic catatonia, a sub-phenotype of the schizophrenic psychoses. The disorder is characterized by qualitative hyperkinetic and akinetic psychomotor disturbances through acute psychotic episodes, and debilitating symptoms in the long term with psychomotor weakness, grimacing facial movements, and apathy. Here, we confirm mapping of a major gene locus on chromosome 15q15 in a second genome scan in a new set of four multiplex families. Non-parametric multipoint linkage analyses identified a broad region with a maximum peak of $Z_{all} = 3.91$ ($p = 0.0063$) and $Z_{lr} = 3.04$ at D15S1234 ($p = 0.0013$), satisfying conventional criteria for confirmed linkage. Parametric affected-only analyses gave maximum HLOD score of 1.65 (D15S1234) with an estimated 47% of families linked. Analysis of individual families showed that one large family showed linkage while two other could be clearly excluded, confirming genetic heterogeneity. No other locus reached suggestive levels of significance. Haplotype analysis on chromosome 15 in this and previously linked families placed the susceptibility region to a 11 cM interval between marker D15S1042 and D15S659. Periodic catatonia is the first sub-phenotype of the schizophrenic psychoses with confirmed linkage despite existence of considerable genetic heterogeneity.

Family-based and case-control studies identify BDNF as a risk locus for bipolar disorder. *P. Sklar^{1,2}, J. Smoller¹, M. Freedman¹, M. Daly², A. Tahl², MG. McInnis³, P. Bennett⁴, Y-M. Lim⁵, G. Kirov⁶, I. Jones⁴, M. Owen⁶, J. Rosenbaum¹, G. Sachs¹, N. Craddock⁴, JR. DePaulo³, ES. Lander².* 1) Psychiatry, Harvard Medical School, Boston, MA; 2) Whitehead Institute Genome Center, Cambridge, MA; 3) Johns Hopkins University, Baltimore, MD; 4) University of Birmingham, Birmingham, UK; 5) Columbia University, NY, NY; 6) University of Wales, Cardiff, UK.

Previous data has identified an allele of the gene BDNF as a risk factor for bipolar disorder in a sample of 136 proband-parent trios. Excess transmission of Val66Met SNP located in the proBDNF protein was detected (T/U, 53/34, $p=0.042$). We now report replication samples that provide independent confirmation of the association between this SNP and bipolar disorder. The first replication sample consisted of 197 parent-proband trios (NIMH samples). Genotyping of Val66Met reveals replication of the original association between Val66 and bipolar disorder with a T/U ratio of 75/56, $p=0.048$. Genotyping in an additional 145 parent-proband trios from the UK demonstrated a trend in the same direction (T/U=38/32, $p=0.17$). In a case-control study of 122 bipolar patients and 122 controls the Val allele was more frequent as expected (Val allele=81.4% cases, 77.9% controls). A genomic control analysis of 37 randomly distributed, unlinked markers revealed no systematic stratification between the cases and the controls. A pooled P value of 0.022 for the family-based and case-control replication samples was obtained by calculating the expected mean and variance of the number of risk alleles found in cases given the number of samples analysed. Under a multiplicative model, the transmission ratio and the odds ratio are estimators of the genotypic relative risk. A pooled value was obtained for all samples of 1.33. In summary, we present data from 600 bipolar probands indicating that the Val66 allele is a risk allele for bipolar disorder. These data are supported by haplotype analyses identifying an undertransmitted haplotype uniquely marked by the rare Met66 allele in all patient samples studied that may be protective for bipolar disorder.

A fifth locus implicated in Autosomal Dominant Hypercholesterolemia. *D. Allard*¹, *M. Abifadel*^{1,2}, *JP. Rabès*^{1,3}, *L. Villéger*¹, *M. Varret*¹, *A. Derré*¹, *C. Junien*^{1,3}, *C. Boileau*^{1,3}. 1) INSERM UR383, Necker-Enfants Malades Hospital, Paris, France; 2) Saint-Joseph University, Beirut, Lebanon; 3) Ambroise Paré Hospital, Paris, France.

Autosomal Dominant Hypercholesterolemia (ADH) is one of the most common hereditary diseases characterized by a selective increase of LDL particles, giving rise to premature mortality from cardiovascular complications. Most of the cases of ADH are caused by mutations in the Low Density Lipoprotein Receptor gene (LDLR) or in the gene encoding its ligand: apolipoprotein B (APOB). On going work in our laboratory has shown that mutations in at least two other genes (HCHOLA3 and HCHOLA4) also account for ADH. By linkage analysis, we excluded the involvement of the APOB and LDLR genes in a new large French pedigree (HC92). HC92 comprises three generations. Among the 35 family members who gave informed consent and were investigated, 15 are affected. We tested 8 markers for the HCHOLA3 region and 3 markers for the HCHOLA4 region. No common haplotype segregation with the disease was found in these two genomic regions. We concluded that linkage was excluded between the disease and both these loci. Consequently, we hypothesize that the ADH phenotype of this pedigree could be due to another new locus. In conclusion, our results show that the ADH phenotype is far more heterogeneous than previously assumed and that mutations in 5 genes are involved.

A Promoter Polymorphism of 5-Lipoxygenase is Associated with Carotid Atherosclerosis in the Human

Population. *H. Allayee*¹, *J.H. Dwyer*², *J. Fan*², *K.M. Dwyer*², *R.A. Mar*¹, *A.J. Lusis*³, *M. Mehrabian*³. 1) Department of Human Genetics, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095; 2) Department of Preventive Medicine, USC Keck School of Medicine, Los Angeles, CA 90033; 3) Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095.

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. Using a knockout mouse model, we previously demonstrated that 5-lipoxygenase (5-LO) confers dramatic resistance to atherosclerotic lesion formation and decreases fasting plasma insulin, even in the presence of highly elevated cholesterol levels. To test whether 5-LO is associated with atherosclerosis and insulin levels in the human population, we genotyped a promoter polymorphism, consisting of a variable number of tandem Sp1/Egr-1 binding sites, in 460 individuals phenotyped for carotid intima-media thickness (IMT). The results demonstrate that individuals who are homozygous for the deletion alleles of the polymorphism have significantly higher IMT ($.740 \pm .122\text{mm}$ vs. $.650 \pm .093\text{mm}$; $P < .0003$) than those individuals with genotypes of either wildtype or addition alleles. These results remained statistically significant ($P < .04$) even after adjustment for multiple risk factors, including age, lipid levels, blood pressure, and medication. Furthermore, the same individuals with deletion alleles also exhibited significantly higher ($P < .0008$) fasting plasma insulin levels. These findings are consistent with those observed in the mouse and demonstrate that genetic variation in the 5-LO gene is associated with surrogate markers of CAD in humans as well. Importantly, the effect on IMT is only partially mediated by conventional biologic risk factors, which suggests that 5-LO is a strong risk factor for atherosclerosis. Additional studies with other mouse models and human populations will be required to replicate these findings and may help to determine the functional basis for this association.

Discovery of genetic markers for myocardial infarction risk. *B. Jordan¹, K.L. Lunetta¹, M.A. Varlaro¹, L. Yan², P.A. Jordan¹, F. Kelley¹, F. Lysholm², R. Sanders², J. Dunker², S. Toth², K.G. Ardlie¹.* 1) Genomics Collaborative, Inc., Cambridge, MA; 2) Pyrosequencing, Inc., Westborough, MA.

Establishing a genetic risk profile for myocardial infarction (MI) could be a valuable tool in prescribing preventative treatment tailored to individual patients. Many genes in different pathways are likely to contribute to risk for this complex trait. We chose to investigate genes in the renin-angiotensin-aldosterone system (RAAS) because they play a key role in regulating blood pressure, which in turn affects cardiovascular disease risk. The RAAS pathway has also been implicated in heart failure, MI, and ventricular remodeling after MI. Previous studies of the association between RAAS gene polymorphisms and MI risk have had mixed results, and have typically been assessed on small sample populations, so further study with a larger sample size is warranted. We investigated 12 single nucleotide polymorphisms (SNPs) in three RAAS genes based on initial findings from an unpublished Eureka Medical study: angiotensinogen (AGT), angiotensin converting enzyme (ACE), and angiotensin II receptor type 1 (AGTR1) in a large sample of US and Polish Caucasians. We genotyped 693 non-diabetic patients who had suffered an MI (370 of which also have hypertension), 396 patients with type 2 diabetes and an MI, 658 patients with hypertension but no MI, and 659 healthy controls. We tested individual SNPs and haplotypes across each gene for association with MI risk and other outcomes. No single SNP was significantly associated with MI risk after correction for multiple tests, although two AGTR1 SNPs were significant predictors of MI among hypertensives at the nominal .05 level. In addition, haplotypes for all three genes showed suggestive evidence for association with MI risk among hypertensives.

A high-throughput SNP typing system for genome-wide association studies in patients with myocardial infarction. *Y. Ohnishi, T. Tanaka, K. Ozaki, A. Sekine, Y. Nakamura.* SNP Research Ctr, RIKEN, Tokyo, Japan.

SNPs are useful markers for identifying genes responsible for and/or associated with common diseases and for directing personalized medical care. Furthermore, because they are so frequent in the genome and can be genotyped quite easily, SNPs can serve as markers for a whole genome association study. However, one of the most difficult issues to be solved for the whole-genome association study using SNPs is reduction of the amount of genomic DNA for genotyping. The presently-available technologies require too large quantity of genomic DNA to be practical. To overcome this problem, we combined the Invader assay with multiplex PCR performed in the presence of Taq polymerase antibody as well as a novel 384-well card system that reduced a reaction volume. We amplified 96 genomic DNA fragments simultaneously in a single tube, and analyzed each SNP by the Invader assay. Since we used 10-20 nanogram of genomic DNA as a template for multiplex PCR, the amount needed to assay one SNP was only 0.1-0.2 nanogram. Our results strongly indicate the feasibility of undertaking genome-wide association studies using blood samples of only 5-10 milliliter. Using these technologies, which allow us to perform as many as 450,000 typings in one day, we had already screened 60,000 SNPs at present. As a result, we had identified some loci where significant association ($p < 10E-4$) was observed. We made a dense SNP map in those loci, and then myocardial infarction related genes were identified. Now we perform the functional analysis about these genes.

Linkage analysis between DNA markers on human chromosome 13q and factor VII levels in families of patients undergoing coronary arteriography. *S.H. Jee^{1,3}, J.E. Lee², Y. Jang³, T.H. Beaty⁴*. 1) Graduate School of Health Sci., Yonsei University, Seoul, Seoul, South Korea; 2) DNA Link, Inc, Seoul, Korea; 3) Cardiovascular Genome Center, Seoul, Korea; 4) Johns Hopkins University, MD, USA.

An elevated plasma level of factor VII is a risk factor for coronary artery disease. However, in general, the genetic studies of factor VII have identified alleles associated with low levels and not high levels of factor VII activity, and R353Q on 13q34 is a good example. The aim of this study was to determine whether 13q region contains specific loci that affect elevated plasma factor VII activity or not. Maximum likelihood methods were used to fit several genetic and non-genetic models of inheritance to these data to determine whether an unobserved Mendelian major gene could explain the familial distribution of factor VII in 661 family members of 112 probands who underwent elective coronary arteriography. DNA polymorphisms, R353Q, were determined. A genomic scan to detect loci related to factor VII was conducted in 29 extended pedigree (126 individuals). We selected 6 polymorphic microsatellite markers (D13S1265, D13S1315, D13S261, D13S285, D13S1295, and D13S293) on chromosome 13q. Factor VII activities were adjusted for age, gender, body mass index, smoking, alcohol consumption, menopause status, and triglycerides prior to this segregation analysis. Adjusted factor VII activities showed strong familial aggregation with an estimated parent-offspring correlation of 0.34, sibling correlation of 0.36 and a smaller spouse correlation of 0.16. Mendelian single-locus models with either two or three underlying genotypic distributions of factor VII activities were best supported by these data. In linkage analysis, a putative major gene with a codominant allele for elevated factor VII activity gave evidence against linkage (LOD score of 4.19 at D13S285 with a recombination fraction from 0.001). The segregation analysis showed that factor VII was under strong genetic influence, following the Mendelian model. However, factor VII showed no linkage to the 13q region, indicating the major gene effect influence in factor VII was not explained by these loci.

Exclusion of chromosome 2 and 14 coronary artery disease loci in an American Caucasian population. *G. Shen*^{1, 2, 3}, *S. Rao*^{1, 2, 3}, *R. Cannata*^{1, 2, 3}, *E. Zirzow*^{1, 2, 3}, *A. Bakos*^{1, 2, 3}, *E. Topol*^{1, 2, 3}, *Q. Wang*^{1, 2, 3}. 1) Department of Cardiovascular Medicine; 2) Department of Molecular Cardiology; 3) Center for Molecular Genetics; Cleveland Clinic Foundation, Cleveland, OH 44195, USA.

Coronary Artery Disease (CAD) and Myocardial Infarction (MI) are the leading cause of mortality and morbidity in the developed countries. In the United States, 12.6 million people are affected with CAD, 7.5 million people develop MI, and over half a million people die from CAD each year. CAD is believed to be caused by interactions between genetic and environmental factors. Two genome-wide scans, one in the Finnish population and the other in the German population, have produced evidence for linkage of CAD to D2S129 on chromosome 2q21.1-q22 and D14S1426 on 14q, but the responsible genes remain to be cloned. To evaluate these findings, we studied an American Caucasian population (GeneQuest) consisting of 2030 individuals from 428 families with premature CAD (atherosclerotic CAD occurring to males of < 45 years of age and females of < 50 years of age). The average family size was 4.7 members, and relevant clinical, physiological and biochemical data were obtained. The GeneQuest population contained 1303 sibling pairs. We genotyped markers D2S129 and D14S1426 in our GeneQuest population with an ABI 3100 Genetic Analyzer. The genotyping data was analyzed using Haseman-Elston regression. No significant evidence was observed for linkages of the GeneQuest population to chromosome 2q21.1-q22 and 14q CAD loci. A P-value of 0.411 was obtained for marker D2S129 and a P-value of 0.589 was found for marker D14S1426. These results suggest that the susceptible gene(s) in the GeneQuest population may be different from that in the Finnish and German populations.

A whole genome linkage approach identifies a novel ApoA1 mutation causing five percent of familial hypoalphalipoproteinemia in the Dutch population. *M. Samuels¹, G.K. Hovingh², J.A. Kuivenhoven², M.-P Dube¹, H. Verschoof¹, Y.P. Goldberg^{1,3}, J.J.P. Kastelein², M.R. Hayden^{1,3}, A. Brownlie¹.* 1) Xenon Genetics, Inc., Burnaby, BC, Canada; 2) Academic Medical Ctr, Univ of Amsterdam, Amsterdam, Netherlands; 3) Dept of Medical Genetics, Univ of British Columbia, Vancouver, BC, Canada.

The regulation of HDL-cholesterol in humans is of great therapeutic interest, given that HDL-C has been shown to correlate with risk for coronary artery disease (CAD). We have taken a family-based linkage approach to identify genes controlling HDL-C levels. As part of an effort to identify novel genes, we conducted a genome scan of approximately 800 genetic markers on non-ABCA1 families of Dutch origin. We used linkage and haplotype analysis to identify regions of the genome that co-segregated with disease. In one Dutch family, a multipoint LOD score of 3.8 was observed on chromosome 11. Subsequent haplotype analysis confirmed perfect segregation of a 13cM interval, spanning the ApoA1 gene, in this family. Sequencing the ApoA1 coding region in genomic DNA from affected individuals revealed a novel point mutation resulting in the amino acid substitution L178P. The mutation segregated on the affected haplotype, consistent with the low HDL-C phenotype in the family, and was not observed in 374 control chromosomes. Among 94 additional Dutch individuals with low HDL-C, 5 more carriers were identified (5.3%). These individuals occurred in extended pedigrees in which the variant also cosegregated with the phenotype. In all, a total of 33 mutation carriers were identified, providing an unprecedented study group for analysis of biochemical phenotypes and CAD risk. The L178P variant was shown to be significantly associated with low HDL-C, based on comparison of the 33 mutation carriers to 48 related non-carrier controls ($p < 0.0001$). In those carriers over the age of 20, a significant effect of the mutation on CAD was noted, as 9/30 carriers were affected with CAD as compared to 2/83 related non-carrier controls ($p = 0.00048$). These results demonstrate the ongoing value of linkage approaches to complex disease.

Association of the PIA polymorphism with platelet activation following coronary stent implantation. *C. Stranieri¹, E. Trabetti¹, D.J. Angiolillo², A. Fernandez-Ortiz², E. Bernardo², C.F. Barrera², M. Viveros², M. Sabate², C. Macaya², P.F. Pignatti¹.* 1) Sect Biol & Genetics, Mother-Child, Biol & Genetics, Verona, Italy; 2) Cardiovascular Institute-San Carlos University Hospital, Madrid, Spain.

The PIA polymorphism (Leu33Pro) of the GPIIIa gene has been suggested to play an important role in coronary thrombosis. In vitro studies have shown differences in platelet sensitivity towards antiplatelet drugs related to this polymorphism, suggesting a pharmacogenetic role. The aim of the study was to assess the influence of PIA polymorphism on platelet activation following a loading dose of clopidogrel in pts undergoing coronary stent implantation (CSI). Platelet activation was assessed as GPIIb/IIIa (anti-Fibrinogen-FITC) expression in platelets stimulated with 2M ADP using whole blood flow cytometry at baseline, 10 min, 4h, and 24h, following CSI. Genotype distribution in 32 pts was: 26 pts (81%) homozygous PIA1/PIA1 and 6 (19%) heterozygous PIA1/PIA2. A similar distribution was observed in 42 healthy control volunteers: 32% PIA1/PIA2. GPIIb/IIIa activation (expressed as percentage of positive platelets) was higher in PIA1/PIA2 pts at all time points ($p=0.03$). Clopidogrel reduced platelet activation following the fourth hour after CSI in PIA1/PIA1 pts up to 36,25% more than PIA1/PIA2 pts ($p=0.04$). No differences in fibrinogen level, platelet count, risk factors, and clinical status were observed between groups. Conclusion: The PIA polymorphism of the GPIIIa gene seems to be associated with different patterns of platelet activation in pts with coronary disease. In particular, PIA1/PIA2 pts undergoing CSI may have a greater GPIIb/IIIa activation and a lower response to clopidogrel than PIA1/PIA1 pts. If these preliminary data will be confirmed in a larger sample, different antithrombotic treatments could be suggested after genotyping for the PIA polymorphism.

Association & linkage study of ADHD using a polygenic model. *L. Park*¹, *K. Nummy*², *C. Huang-Pollock*³, *M. Rappley*⁴, *J. Nigg*³, *K. Friderici*². 1) Genetics Program; 2) Microbiol. & Mol. Gen; 3) Dept. of Psychology; 4) Peds/Hum. Dev.; Michigan State University, East Lansing, MI.

Attention-deficit hyperactivity disorder (ADHD) is a heritable disorder characterized by inappropriate inattention, hyperactivity and impulsiveness in children that is now known to persist into adulthood in a substantial percentage of cases. A polygenic model probably best explains the inheritance of ADHD, and there has been excitement in the field about reported positive effects for single candidate genes. Yet few replicated studies associate ADHD with specific genes, especially in adults. Because patients respond positively to psychostimulant medications, variants of genes involved in dopamine and serotonin function have been most often investigated as risk alleles for ADHD. We typed 5 genes in both adult ADHD (14 control, 31 affected) and childhood ADHD (33 control, 42 affected). Diagnostic criteria for ADHD were directly evaluated using standardized rating scales to assess DSM-IV criteria, severity, onset, duration, and impairment. Previously reported polymorphisms were analyzed for the dopamine transporter, dopamine D4 receptor, serotonin transporter, and 5-hydroxytryptamine receptor. In addition we examined the α -2A-adrenergic receptor (ADRA2A) as a candidate. For ADRA2A, 7 SNPs in the regulatory region of the gene were identified and the 3 which were present in the population at an allele frequency of >10% were analyzed for linkage. None of the five genes had highly significant association or linkage with ADHD using TDT and case-control methodologies. However, one of the SNPs in the promoter region of ADRA2A locus showed association with ADHD in the adult sample. These data support other results in suggesting that when ADHD is carefully assessed using DSM-IV criteria, individual catecholaminergic genes likely to be contributing to ADHD symptoms may be expected to contribute only a small component to the overall constellation of symptoms that comprise this phenotype. Large sample sizes, definition of behavioral and cognitive subtypes, and use of cognitive and physiological endophenotypes are recommended to evaluate genetic contributions to ADHD.

Apparent uniparental disomy in a familial case of autism spectrum disorder. *A.L. Haefele¹, D.A. Figlewicz¹, C.J. Stodgell²*. 1) Neurology, University of Rochester, Rochester, NY; 2) OB/GYN, University of Rochester, Rochester, NY.

Autism spectrum disorders (ASDs) are known to have a strong genetic etiology. Recent genome-wide scans have suggested that one, if not more of these responsible genes reside on chromosome 7q. This suggestion has been supported by candidate gene studies that have associated genes found on Chr. 7q (e.g., WNT2, RELN, and FOX2P) with autism and language disorders. This study screened 60 pedigrees with at least one person having an ASD for sharing of alleles on 9 genetic markers and the gene RELN. These markers were spaced, on average, every 10 cM starting at the centromere, and included regions close to those where significant LOD scores were found in other studies. At this point there is no evidence for significant linkage at these markers. However, based on the genotypes of the markers examined, there appears to be a uniparental disomy in one of the pedigrees. In the pedigree, the proband shares the same genotype with the mother starting at marker d7s1843 and continuing to marker d7s1826 where her sister, who has a language delay, appears to have only one of her father's alleles. These data suggest that one of the genes important to the ASD phenotype may be located close to d7s1826 and d7s1826. Supported by NICHD PO125466, a Collaborative Program of Excellence in Autism.).

Vervet monkey (*Chlorocebus aethiops*) as primate model for the genetic study of complex traits - reconstruction of a complete pedigree structure of a captive vervet colony. R.A. Ophoff^{1, 2}, Y. Wang¹, M. Tampilic¹, J. Bailey³, M. Jorgensen³, L. Fairbanks³, N.B. Freimer^{1, 2, 3}. 1) UCLA Center for Neurobehavioral Genetics, Dept of Psychiatry, UCLA, Los Angeles, CA; 2) Dept of Human Genetics, UCLA, Los Angeles, CA; 3) Neuropsychiatric Institute, UCLA, Los Angeles, CA.

The vervet monkey, also known as the African Green monkey, is widely used in biomedical research. A captive population at the UCLA-VA Vervet Research Colony (VRC) contains over six hundred normally reared animals in an extended six-generation pedigree founded by a limited number of captured animals from St. Kitts (West Indies) between 1975 and 1987. Phenotypes relevant to a number of different psychiatric and medical disorders have been assessed in all colony members, using consistent and reliable protocols. Preliminary heritability analyses have found significant genetic contributions to these traits. Our goal is to develop genetic resources that will allow this colony to be used to identify susceptibility loci for a number of complex (behavioral) traits.

As a first step in our efforts we developed a panel of informative microsatellite markers to be used for paternity testing and extension of the pedigree of the VRC. We tested 86 human highly-polymorphic fluorescently-labeled microsatellite markers that were used successfully for previous human mapping projects. Applying a standard PCR protocol (with low stringency conditions), about 60% of the markers amplified successfully and 34 of these markers revealed 2 alleles in eight random vervet subjects from the colony. From these amplicons we selected the 16 most informative markers that amplified well with scoreable and reproducible alleles. Genotyping of all available subjects from the colony (n=624) revealed an average of 4.8 alleles per marker. Statistical analysis is underway to verify and complete the pedigree structure of the colony. Once the pedigree structure of the vervet colony is confirmed we will generate a vervet genetic map.

Program Nr: 1555 from 2002 ASHG Annual Meeting

Genetic study of inherited Human Disease using a new mutant mouse, Sims (Sexual Immaturity, Megaencephaly, and Seizure): phenotype and chromosome mapping for positional cloning. *b. oh¹, s. koo², s. jin¹, d. park¹, k. lee², s. jung², k. kimm¹*. 1) Genome Research Institute, National Institute of Health, Seoul, Korea; 2) Division of Genetic Disease, National Institute of Health, Seoul, Korea.

Among the experimental animals, mouse has been highlighted as an excellent model system because mouse, one of the smallest mammals, is similar in development, genetics, and physiology to human. In addition the genetic and developmental information in mouse have been accumulated to make the study easier and faster than any other mammals. Sims, a new mutant mouse has a mild tremor and seizure and are sterile in either sex. The uterus in the affected female and seminal vesicle in the affected male are smaller than their normal litters and there is no detectable testosterone in the serum of the affected male. In addition the brain of the mutant is 30% heavier than that of littermate at their 3 month-old, probably because it continuously grows even after the puberty in the mutant mouse. Intraspecies cross to CAST/Ei was carried out and the 37 affected mice was analyzed for the linkage and the gene was mapped on chromosome 18, 20 cM from the centromere. To identify the gene, the fine genetic map and physical map of sims has been performed to obtain the 0.5Mb contig of the region, and the candidate genes located in the region have been identified.

Genetic Origins in a South American Clefting Population. *A.R. Vieira¹, J.C. Karras², I.M. Orioli³, E.E. Castilla⁴, J.C. Murray¹.* 1) Dept Pediatrics, Univ Iowa, Iowa City, IA; 2) College of Dentistry, Univ Iowa, Iowa City, IA; 3) Dept Genetics, UFRJ, Rio de Janeiro, Brazil; 4) Dept Genetics, Fiocruz, Rio de Janeiro, Brazil/CONICET, Buenos Aires, Argentina.

It has been proposed that susceptibility to clefting in South America is related to Amerindian ancestry where clefting has a higher frequency than the other admixed populations (Caucasian and African) that make up the diverse racial mix of current South Americans. To clarify the genetic origins in and establish a method for genetic mapping a South American population affected with clefting had mitochondrial DNA variation and Y chromosome markers studied. 217 subjects and matched controls were selected through Latin-American Collaborative Study of Congenital Malformations (ECLAMC). The case group showed a higher frequency of Native American haplogroups and a lower frequency of African haplogroups ($p < 0.00001$). Also, the case group showed a much higher frequency of the specific native American haplogroup D than the control group ($p < 0.00001$). For the Y chromosome markers, the case group showed a lower frequency of the African-specific marker YAP ($p = 0.002$) and a higher frequency of the Native American-specific marker DYS199 ($p < 0.00001$). Even though there are differences found in the frequencies of the markers studied, the contribution of each founder population is similar for both groups. Results suggest a strong Native American maternal contribution and a strong Caucasian (Spanish and Portuguese) paternal contribution to the population studied. The implications of this finding include the possibility of using admixture mapping approaches to this population.

The gene for Juvenile Hyaline Fibromatosis maps to chromosome 4q21. *S. Hanks*¹, *M. Dunstan*², *M.D. Teare*³, *A. Futreal*⁴, *F.M. Pope*⁵, *N. Rahman*¹. 1) Section of Cancer Genetics, Institute of Cancer Research, Sutton, Surrey, UK; 2) Dept of Psychological Medicine, University Hospital of Wales College of Medicine, Cardiff, UK; 3) Mathematical Modelling and Genetic Epidemiology Group, University of Sheffield, Sheffield, UK; 4) Cancer Genome Project, The Wellcome Trust Sanger Institute, Hinxton, Cambs, UK; 5) Connective Tissue Matrix Genetics Group, Division of Life Sciences, Kings College London, UK.

Juvenile Hyaline Fibromatosis (JHF) (MIM 228600) is an autosomal recessive condition that typically presents in the first few years of life with nodular skin lesions, on the scalp, face and hands. The distribution and burden of lesions tends to increase throughout childhood, often necessitating recurrent excision. Gum hypertrophy typically develops and may also require excision. Progressive flexion contractures of joints increasingly limit movement and may result in individuals becoming wheel-chair bound in early adulthood. Diagnosis is confirmed by skin histology which typically shows an abundance of a homogenous, amorphous hyaline material. We performed a genome-wide linkage search in two families with JHF from the same region of Gujerat and identified a region of homozygosity on chromosome 4q21. Dense microsatellite analyses within this interval in five JHF families from diverse origins demonstrate that all are compatible with linkage to chromosome 4q21 generating a multipoint LOD score of 5.5. Meiotic recombinants place the gene for juvenile hyaline fibromatosis within a 7cM interval bounded by D4S2393 and D4S395. The minimal interval contains 19 known genes and 47 predicted genes. Of the known genes, a number are potentially of interest as candidates, including Bone morphogenetic protein 3 (*BMP3*), Fibroblast growth factor 5 (*FGF5*) and the homolog of mouse BMP-2 inducible kinase (*BIKE*).

Program Nr: 1558 from 2002 ASHG Annual Meeting

An SNP study of candidate genes in Cleft Lip and Palate. *R. Ingersoll¹, S. Hiebler¹, M. Campbell¹, J. Barton¹, F.W. Schuler¹, L. Kasch¹, I. McIntosh¹, T. Beaty², A.F. Scott¹.* 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine; 2) Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University.

We are investigating the genetics of non-syndromic cleft lip and palate by identifying single nucleotide polymorphisms (SNPs) in candidate genes identified by linkage analysis, animal models or presumed function. SNPs are identified in coding region, 3' UTR and promoter sequences by PCR amplification and direct sequencing of DNAs from unrelated parents of 15 probands. We are taking advantage of the increasingly complete human genome sequence and NCBI resources such as OMIM, LocusLink and Map Viewer to identify genes and genomic sequence. Sequences are aligned manually and visually inspected for differences. As of June 2002, PCR primers have been designed for 564 amplicons from 48 genes. Fifteen genes have been completely sequenced (156 amplicons) and 189 amplicons from an additional 16 genes have been studied from other genes. A total of 375 SNPs have been identified of which 86 (23%) occur in exons and 41 (48% of cSNPs; 11% of total SNPs) of which result in amino acid changes (nsSNPs). Allele frequencies will be determined for each SNP in a collection of trios obtained through a proband with a non-syndromic oral cleft (cleft lip with or without cleft palate and cleft palate only). Previous studies of these case-parent trios have shown evidence of linkage in the presence of disequilibrium for several candidate genes, in particular MSX1 and TGFB3. Both of these genes have been screened and a number of novel SNPs were identified. To date, 150 SNP assays have been developed for the Illumina bead-array platform and 253 CLP samples genotyped.

Identification of Type 1 Diabetes linked genes on chromosome 21. *R. Bergholdt, J. Nerup, F. Pociot.* Steno Diabetes Center, Gentofte, Denmark.

Background and Aim: In a recent genome wide linkage analysis for Type 1 Diabetes Mellitus (T1DM)-susceptibility loci in 408 Scandinavian multiplex families, a newly identified locus on the distal part of chromosome 21, especially revealing linkage to T1DM in the Danish population with a maximum LOD score of 2.33 ($P=0.009$), was identified. The linked region on chromosome 21 comprises 20 Mb, which in this region equals approximately 35 cM. The aim of the study was to characterize and fine-map a genetic region on chromosome 21 showing linkage to T1DM.

Material and methods: DNA from 155 Danish T1DM sib-pair families were used, all probands with age at onset below 30 years. 20 microsatellite markers were chosen, covering the 35 cM region on chromosome 21. The 155 sib-pair families were typed for all 20 markers. Collection and allele-calling of data were done using the software programs GeneScan, GenoTyper and GeneMapper, followed by multipoint LOD score analysis of all the markers using GeneHunter. Genotyping of SNPs were performed by RFLP-PCR or MS-PCR.

Results: Multipoint LOD score analysis of 20 new microsatellite markers and the 7 chromosome 21 markers used in the scandinavian genome-scan, in a Danish collection of 155 sib-pair families, revealed two separate loci, showing linkage to T1DM. For locus 1, covering 4 cM, a peak LOD score of 3.19 ($P=0.0005$) was obtained, whereas locus 2, covering approximately 3 cM, had a peak LOD score of 2.83 ($P=0.0018$). The regions below the peaks are analyzed for candidate genes. 4 known SNPs in coding regions of the CBR1 gene and 1 SNP in the CBR3 gene, have been typed, none revealing T1DM association. Furthermore the two most common mutations in the AIRE gene, coding for APECED (autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy), has been examined in 100 diabetic patients, no mutations were identified.

Conclusion: Fine-mapping of a T1DM linked region on chromosome 21 has narrowed the linked region into two separately linked regions with peak LOD scores of 3.19 and 2.83, respectively. This suggests that one or several T1DM linked genes exist in the region.

GENETIC HETEROGENEITY IN AUTOSOMAL DOMINANT ALPORTS SYNDROME (ADAS). *F. Boaretto¹, S. Savoldi³, D. Santon¹, S. Crovella^{1,2}, M. Martone³, M. Artero³, M. Pennesi², G. Panzetta³, A. Amoroso^{1,2}.* 1) Medical Genetics, University of Trieste, Trieste, TS, Italy; 2) IRCCS Burlo Garofolo, Trieste, TS, Italy; 3) Nephrology and Dialysis Service, Cattinara Hospital, Trieste, TS, Italy.

Alports Syndrome (AS) is a genetically heterogenous glomerulonephritis (GN) with X-linked (for mutations of the COL4A5 and/or COL4A6 genes), autosomal recessive (for alterations of the COL4A3 and/or COL4A4 genes), and autosomal dominant (AD) forms. ADAS is due to mutations in the COL4A3 and/or COL4A4 genes or, when associated with a platelet defect (Fechtner's Syndrome), to mutations in the MYH9 gene. We studied a 3 generation family affected by GN with microhematuria, dominant transmission, and unfavorable prognosis (2 members in ESRD), in which AS presented also in the pediatric age range. Renal biopsy, performed in 2 patients, showed normal glomerular cellularity without sclerosis in LM; Ig and C3 deposits were absent in IF. EM, performed only in the proband, demonstrated alterations of the GBM consistent with AS. Neurosensory deafness was absent in all subjects examined; ophthalmologic studies in 5 affected members revealed modest lenticular opacities in a single pediatric patient. Macrothrombocytopenia, lipid abnormalities and hypertension, which have been previously described in ADAS, were absent in the present study. We analysed 11 members (9 affected and 2 unaffected) after informed consent. In order to exclude the known loci, we used published markers D1S498, D1S305 (locus ADAS with hypertension), D2S396, D2S159, D2S2345, D2S126, D2S277 (COL4A4\4A3), D2S283, D2S426, D2S272 (MYH9). The two-point linkage analyses were performed with the computer program MLINK from the LINKAGE Package (Ver. 5.2), assuming a penetrance of 0.95, AD transmission, no recombination sex difference and gene frequency of 1/10.000. We obtained negative LOD scores with values <-2 for about all markers analysed, at recombination fraction $\theta=0$. These results further confirmed the genetic and clinical heterogeneity of ADAS and suggested that other GBM components may be involved in the pathology, codified by as yet unidentified genes, which a genome-wide study, currently in progress, may localize.

Further evidence for linkage of specific language impairment to 13q21 from an American sample. *C.W. Bartlett¹, J.F. Flax¹, M.W. Logue³, V.J. Vieland³, P. Tallal¹, L.M. Brzustowicz^{2,4}*. 1) Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ; 2) Department of Psychiatry, University of Medicine and Dentistry New Jersey, New Jersey Medical School, Newark; 3) Department of Biostatistics, Division of Statistical Genetics, College of Public Health and Department of Psychiatry, College of Medicine, University of Iowa, Iowa City; 4) Department of Genetics, Rutgers University, Piscataway, NJ.

Specific language impairment (SLI) is characterized as a failure to develop language normally, in absence of explanatory factors such as low intelligence, inadequate educational opportunity, hearing impairments or neurological difficulties. Previously, we demonstrated linkage of SLI to 13q21 in five Canadian families of Celtic ancestry with a HLOD of 3.92 under a recessive mode of inheritance with a reading impairment phenotype (Bartlett et al 2002, AJHG). In attempt to replicate these results in a different sample, we have genotyped a dense linkage map of the 13q21 region with a set of 22 American nuclear and extended families (N = 279) ascertained for at least two affected pedigree members. The maximum two-point HLOD score was > 1.5 using the same phenotype and genetic parameters that gave the strongest evidence for linkage in the Canadian dataset. Using both samples, HLOD for multipoint linkage was 4.92. To further characterize the strength of evidence in the region, we used the posterior probability of linkage to combine the datasets. The PPL is specifically designed to accumulate linkage evidence across datasets in a statistically rigorous way. Based on the original set of families, the PPL was 53% at D13S1317; after updating with our current data the PPL at this marker is 93% (preliminary calculation). These data indicate that the 13q21 locus segregates not only in Canadian families of Celtic ancestry, but also in American families ascertained for SLI. These results increase the possibility that the 13q21 autism locus found in American families, is the same demonstrated in our SLI sample.

Evidence of Linkage and Association between the Lipoprotein Lipase Gene (LPL) and Insulin Resistance in Hispanic Hypertension Families. *X. Guo¹, L.J. Raffel¹, M.O. Goodarzi^{1,2}, K.D. Taylor¹, A.H. Xiang³, M. Quinones², C. Samayoa¹, W.A. Hsueh², T.A. Buchanan³, J.I. Rotter¹.* 1) Cedars-Sinai Medical Ctr, Los Angeles, CA; 2) UCLA School of Medicine, Los Angeles, CA; 3) USC Keck School of Medicine, Los Angeles, CA.

We have previously shown that blood pressure (BP) and insulin resistance (IR) cosegregate in Hispanic families and that there most likely are gene(s) contributing to both BP and IR. Previous studies have shown evidence of linkage and/or association of the HindIII polymorphism in the LPL gene with IR, as well as IR-associated hypertension, dyslipidemia, and atherosclerosis. However, in most cases insulin sensitivity was assessed by indirect methods. To further examine the role of the LPL gene in IR, we genotyped 390 members of 77 Hispanic families for six SNPs in the 3' region of LPL (7315, 8292, 8393, 8852, 9040, 9712). Families were ascertained via a hypertensive proband and insulin resistance was directly assessed via hyperinsulinemic euglycemic glucose clamps in the adult offspring. Multipoint linkage analyses were performed using SIBPAL2. Association between the six SNPs, LPL haplotypes and IR-related traits were evaluated using the QTDT program. Significant linkage was found with IR as measured by clamp glucose infusion rate (GINF, $p=0.0042$). Applying the population stratification model for haplotype association analysis in QTDT, the combined SNP haplotype 'CAGGCA' was found to be associated with higher GINF (less IR) ($p=0.023$). In further examination of association with each SNP individually, we found that SNP 9712 contributes the most to the association ($p=0.01$); allele 2 (presence of adenine) is associated with less IR than allele 1 (presence of guanine). These results support a direct role for the LPL gene in determining insulin resistance, confirming our previous observation of linkage in Mexican American coronary artery disease families. Variation in the 3' end of the LPL gene is most likely involved in determining IR. In addition, we have identified a specific haplotype that is associated with increased insulin sensitivity in this hypertensive Hispanic population.

Refinement of the critical region for Nance-Horan Syndrome to 1.3Megabases on chromosome X. *K.P. Flowers¹, J.E. Craig^{2,3}, D.A. Mackey^{1,2,4}, J.M. Stankovich¹, J.D McKay¹, M.M. Sale^{1,5}.* 1) Menzies Ctr Pop Health Res, Univ Tasmania, Hobart, Australia; 2) Centre for Eye Research, University of Melbourne, Royal Victorian Eye and Ear Hospital, Melbourne, Australia; 3) Dept Ophthalmology, Flinders Medical Centre, Adelaide, Australia; 4) Dept Ophthalmology, Royal Children's Hospital, Melbourne, Australia; 5) Dept Internal Medicine, Wake Forest University School of Medicine, Winston-Salem, USA.

Nance-Horan Syndrome is an X-linked disorder characterised in hemizygous males by bilateral congenital cataract and dental anomalies including screw-driver blade shaped incisors and supernumerary teeth. Female carriers may be affected with mild Y-sutural cataracts, with little or no loss of vision, with or without characteristic dental anomalies. The disorder has previously been linked to a 3.5cM region (5.8Mb) on Xp22.3-p22.1. A large pedigree with Nance-Horan Syndrome was ascertained through the Royal Childrens Hospital, Melbourne, Australia. Linkage analysis was performed at seven markers across a 10cM region using MLINK. The maximum two-point LOD score was 2.2 which is considered significant on the X chromosome. X-linked GENEHUNTER PLUS was used for multipoint and haplotype analysis. A putative shared haplotype was observed between distantly related branches of the family at the two adjacent markers that gave the highest two-point LOD scores. Four novel microsatellite markers, located by searching the draft genome sequence for dinucleotide repeats 15 units or greater in length, were typed to confirm this shared haplotype. The maximum LOD score obtained using all eleven markers was 2.8. Recombinations were observed in two individuals which refine the critical region to a 1.3Mb region on Xp22.3. This is a significant reduction from the previous region and will assist with gene identification for this disorder.

Genome-wide linkage analysis of body mass index across 28 years of the Framingham Heart Study. LD. Atwood¹, N. Heard-Costa¹, LA. Cupples², CE. Jaquish³, PWF. Wilson⁴, RB. D'Agostine⁵. 1) Neurology, Boston Univ Sch Medicine, Boston, MA; 2) Biostatistics, Boston Univ Sch Public Health, Boston, MA; 3) National Heart, Lung, Blood Institute, Bethesda, MD; 4) Medicine, Boston Univ Sch Medicine, Boston, MA; 5) Mathematics, Boston Univ, Boston, MA.

We performed genome-wide linkage analysis of six separate measurements of body mass index (BMI) taken over a span of 28 years from 1971 to 1998 in the Framingham Heart Study. Variance components linkage analysis was performed on 330 families using 401 polymorphic markers. The number of individuals with data at each exam ranged from 1930 in 1971 to 1401 in 1998. Sex, age, and age² were included as covariates in the model.

There was substantial evidence for linkage on chromosome 6 between 139cM and 166cM in the area of *D6S1009*, *GATA184A08*, *D6S2436* and *D6S305*. The six measurements had maximum lodscores of 4.64, 2.29, 2.41, 1.40, 0.99, and 3.08 at 144.4cM, 139.6cM, 141.2cM, 142.8cM, 166.0cM, and 166.0cM, respectively on chromosome 6. There was also evidence for linkage of multiple measures on chromosome 11 between 115cM and 131cM in the area of *D11S1998*, *D11S4464* and *D11S912*. The six measures had maximum lodscores of 0.61, 3.27, 1.30, 0.68, 1.30, and 2.29 at 129.4cM, 119.0cM, 115.0cM, 131.0cM, 115.0cM, and 117.0cM, respectively, on chromosome 11. Both of these regions have been reported in previous studies. Chromosome 8 is the only other chromosome in which at least two datasets had a lodscore greater than 2.0 in the same region. The region containing *D8S1106*, *D8S1145*, and *D8S136* had lodscores of 0.80, 2.78 and 2.29, and 1.01 at 26.0cM, 30.4cM, 30.4cM, and 41.2cM for datasets 1-4. The maximum at 30.4cM is within 10cM of the lipoprotein lipase gene (LPL), which has been linked or associated with BMI or other obesity traits in previous studies.

Evidence in the same regions from multiple measurements indicates that linkage studies of BMI are robust with respect to measurement error. It is unclear if variation in lodscores in these regions is due to age effects, varying sample size, or other confounding factors.

Identification of susceptibility genes in human systemic lupus erythematosus (SLE): Evidence of linkage disequilibrium on chromosome 20. *P.M. Gaffney¹, R.R. Graham¹, W.A. Ortmann¹, P.S. Ramos¹, S.A. Selby¹, P.R. Rodine¹, K.B. Shark¹, K.L. Moser¹, C.D. Langefeld², S.S. Rich², T.W. Behrens¹.* 1) Department of Medicine, University of Minnesota, Minneapolis, MN; 2) Department of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, NC.

SLE is a chronic autoimmune disease characterized by the loss of immunologic tolerance to self-antigens. The clinical manifestation of SLE results from complex interactions between environmental factors, hormonal factors and susceptibility alleles on multiple genes. Our group originally reported linkage at 20p12-13 and 20q11-13 in a collection of 105 sib-pair families with SLE. We now report the results of fine mapping these loci in our family collection of 231 sib pair and 185 trio families. Sixty-four microsatellite markers were genotyped in the regions of 20p12-13 and 20q11-13. The average inter-marker distance was less than 500 kb across both linkage intervals. Genotype data was analyzed using MERLIN, a software package that performs rapid multipoint linkage analysis using sparse inheritance trees and automatically removes unlikely genotypes that can potentially reduce evidence for linkage. The maximal LOD score in the 20p12-13 interval was at marker D20S894 (LOD=2.13, $p=0.0009$). This marker is located approximately 820 kb telomeric from the previously reported best marker in the interval (D20S186, LOD=1.77). The maximal LOD score in the 20q11-13 interval was at marker D20S880 (LOD=1.35, $p=0.006$) approximately 205 kb centromeric to our previously reported best marker (D20S119, LOD=1.64). To investigate evidence for linkage disequilibrium (LD) we used the Pedigree disequilibrium test (PDT). Evidence for LD was found for marker D20S604 (allele 6, $p=0.016$; allele 7, $p=0.034$) within the 20p12-13 linkage interval and markers D20S836 (allele 7, $p=0.018$) and D20S176 (allele 7, $p=0.021$; allele 8, $p=0.002$) within the 20q11-13 linkage interval. Future work will focus on genotyping additional microsatellite and single nucleotide polymorphism markers in the sequence flanking markers D20S604, D20S836, and D20S176. Haplotype based approaches will then be used to better define the regions of LD prior to gene identification.

Genome scan for glaucoma loci in a Costa Rican POAG family. *G. Chavarria Soley*¹, *L. Villalobos*², *C.Y. Mardin*³, *J. Azofeifa*¹, *A. Reis*⁴, *K. Michels-Rautenstrauss*⁴, *B. Rautenstrauss*⁴. 1) INISA, University of Costa Rica, San Jose, Costa Rica; 2) Private Practice, San Jose, Costa Rica; 3) Dep. of Ophthalmology, FAU, Erlangen, Germany; 4) Institute of Human Genetics, FAU, Erlangen, Germany.

Adult onset primary open angle glaucoma (POAG) is one of the major causes of blindness in the world. The disease results in optic nerve damage and a visual field loss, often accompanied by elevated intraocular pressure. Currently 7 loci (GLC1A-GLC1E) and two genes (MYOC, OPTN) are known to be involved in glaucoma. We studied 26 members, 7 patients, from a Costa Rican family with autosomal dominant inheritance of the POAG. After exclusion of MYOC mutations we performed a genome scan with microsatellite markers (mostly Weber panel) with the purpose of identifying the genomic location of candidate gene(s) responsible in the development of glaucoma in this family. The initially calculated two-point lod scores were followed by parametric and non-parametric multipoint linkage analysis. Chromosomes 8 and 9 presented the highest, suggestive two-point lod scores with a value of 2.00 for markers D8S549 and D8S560 and 1.8 for marker D9S1826 (=0 for the three lod scores). For the parametric multipoint linkage analysis location scores higher than 1.50 were found for chromosomes 2, 6, and 8 with values of 1.60, 1.75, and 2.00, respectively. For the non-parametric multipoint analysis the highest scores for the NPL_all statistic are in chromosomes 6, 8 and 12 with values of 1.90, 1.94, and 1.82 respectively. From the known loci only GLC1B (chr. 2) maps to these regions. Mutations in OPTN (chr. 10) are probably excluded, hence this family is promising for the identification of a novel glaucoma candidate gene.

HTR1D and OPRD1 SNPs exhibit significant association to anorexia nervosa. *A.W. Bergen^{1,5}, M.B.M. van den Bree^{1,6}, M. Yeager^{1,5}, J.K. Ganjei¹, R. Welch^{1,5}, K. Haque^{1,5}, S. Bacanu², D.E. Grice³, W.H. Berrettini^{3,4}, C. Bulik⁴, K. Klump⁴, M. Fichter⁴, K. Halmi⁴, A. Kaplan⁴, M. Strober⁴, J. Treasure⁴, B. Woodside⁴, W.H. Kaye^{2,4}.* 1) Biognosis U.S., Inc. (Dissolved); 2) University of Pittsburgh, Pittsburgh, PA; 3) University of Pennsylvania, Philadelphia, PA; 4) The Price Foundation Collaborative Group (www.anbn.org); 5) Core Genotyping Facility, NCI, NIH; 6) University of Wales, Cardiff, UK.

The Price Foundation Collaborative Group (www.anbn.org) has collected a sample of 196 families with two or more relative pairs affected with DSM-IV Anorexia Nervosa (AN) (Kaye et al., *Biol Psych* 2000;47(9):794-803). The family sample has been genotyped with a whole genome linkage panel of microsatellite markers (Weber). The genes for the serotonin 1D receptor (HTR1D) and the opioid delta receptor (OPRD1) are found on chr1p36.3-34.3, a region of the genome observed to be linked to anorexia nervosa (AN) in a linkage analysis of DSM-IV AN diagnosis (Grice et al., *Am J Hum Genet* 2002;70(3):787-792). The entire HTR1D locus and a portion of the OPRD1 locus were evaluated for sequence variation by resequencing. Nine SNPs (four HTR1D SNPs and five OPRD1 SNPs) were chosen for genotype assay development and genotyping was performed on 191 unrelated individuals fulfilling DSM-IV criteria for AN, 442 relatives of AN probands and 98 psychiatrically screened controls. Linkage analysis of these nine SNPs with 33 chr1 microsatellite markers in 37 families with ARPs concordantly affected with restricting AN substantially increased evidence for linkage of this region from an NPL score of 3.45 to 3.91. Statistically significant genotypic, allelic and haplotypic association to AN in the case:control design was observed at both candidate genes with effect sizes for individual SNPs of 2.63 (95%CI=1.21-5.75) for HTR1D and 1.61 (95%CI=1.11-2.44) for OPRD1. In parents and probands only, three SNPs at HTR1D with found to be exhibit significant transmission disequilibrium ($p < 0.05$). The combined statistical genetic evidence suggests that HTR1D and OPRD1 may be involved in the etiology of AN. This abstract does not represent the opinion of the NIH, DHHS, or the Federal Government.

Linkage Analysis in 5 Large Colombian Families with Autosomal Dominant Congenital Cataract. *T. Arguello*¹, *M.L. Tamayo*^{1,2}, *J.C. Prieto*¹. 1) Instituto de Genetica Humana, Universidad Javeriana, Bogota, Colombia; 2) Fundacion Oftalmologica Colombiana, Bogota, Colombia.

Congenital cataract is a clinically and genetically highly heterogeneous eye disorder, with and autosomal dominant (ADCC) inheritance being most common. The genetics of this condition are complex with more than 18 loci for ADCC identified to date. We have investigated a large Colombian population of 5 informative ADCC families. Each family span 4 generations and consisted of more than 20 family members. In all the pedigrees the morphology of the cataract varied in density and severity being the most common the zonular pulverulent, nuclear and posterior polar phenotype. Genotyping and two point linkage analysis were perform to asses linkage to four genes responsible for ADCC localized in chromosomes 1, 2, 21 and 22. The specific regions were mapped using 8 highly polymorphic microsatellites markers (D1S514, D1S2669, D1S2721, D2S117, CRYGPCR1, D21S1446, D22S1028 and D22S315). We have demonstrated linkage to chromosome 1q close to the gene that encodes connexin 50 in one family with positive Lod scores for markers D1S514 ($Z_{max}=2.74$, $max=0.0$) and D1S2669 ($Z_{max}=3.38$, $max=0.0$). Three point analysis gave a maximum Lod score of 18.17 ($max=0.01$) between these two markers. In addition, we have excluded linkage to the other regions with markers D2S117 ($Z_{max}=-2.36$, $max=0.01$) CRYGPCR1 ($Z_{max}=-10.2$, $max=0.01$) D21s1446 ($Z_{max}=-4.04$, $max=0.01$) and D22S315 ($Z_{max}=-3.32$, $max=0.01$). The cataract showed a wide intrafamilial variation, although the majority of affected individuals had pulverulent and polar posterior cataract. In the remaining families we have excluded linkage of the phenotype to these genetic markers with negative Lod scores for all of them. In conclusion, our results indicate that there is a considerable genetic heterogeneity; therefore, to elucidate the mechanism underlying the presence of the ADC, further linkage studies using closed linked polymorphic markers are first necessary to identify if the phenotype of the ADCC in these Colombian families is due to the known cataract loci described in the worldwide population, or if it correspond to a new genetic form.

Genome scan of familial abdominal aortic aneurysms: a candidate region. *S.G. Buxbaum¹, H. Shibamura², J.M. Olson¹, G. Tromp², H. Kuivaniemi^{1,3}.* 1) Dept Epidemiology/Biostat, Case Western Reserve Univ, Cleveland, OH; 2) Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, Michigan; 3) Department of Surgery, Wayne State University School of Medicine, Detroit, Michigan.

Familial aggregation of abdominal aortic aneurysms (AAAs) has been demonstrated. First degree relatives of AAA patients have been shown to have increased risk of AAA with up to 18% of brothers and 5% of sisters having AAA. Population-based ultrasonography screening studies have also emphasized family history as an important risk factor for AAA. Formal segregation studies have demonstrated that AAAs are likely to have a genetic etiology with an autosomal inheritance pattern.

We used a total of 96 families with at least two living members diagnosed with AAA for a two-phase two-stage genome scan. DNA was isolated from blood samples from affected relative pairs (ARPs) and their unaffected relatives, if available. In the Stage I of Phase I we carried out a whole genome scan on 65 affected relative pairs (ARPs). In this scan, five chromosomal regions exceeded our exploratory threshold lod score of 0.8. One region had a lod score of 4.4 with covariate analysis and was selected for further study. In Stage II of Phase I we used additional markers in the region with the highest LOD score and genotyped an additional 121 ARPs at these markers. This gave a total of 186 ARPs (183 full sib pairs and 3 half sib pairs) in the 96 families. In our sample 71% of the ARPs were brother-brother pairs. In a multipoint linkage analysis including the number of affected first degree relatives of the proband as a covariate we found a LOD score of 1.9 using the LODPAL program in S.A.G.E.

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Cosegregation of glaucoma with locus GLC1B (2q12-q13) in a Peruvian family with heterogeneous onset and a recombination within the critical region. *R. Fujita*¹, *M.L. Guevara-Fujita*¹, *R. Perez-Grossman*², *J. Richards*³. 1) Instituto de Genetica y Biologia Molecular, Universidad de San Martin de Porres, Alameda del Corregidor Cdra. 15, La Molina, Lima, Peru. rfujita@amauta.rcp.net.pe; 2) Instituto de Cataratas y Glaucoma, Lima, Peru; 3) W.K. Kellogg Eye Center, U. of Michigan, Ann Arbor, MI, USA.

Glaucoma is a group of ophthalmic disorders having degeneration of the optical nerve as common symptom, with high ocular pressure as a frequent clinical sign. Genetics of glaucoma has developed dramatically and several genes have been localized in different chromosomes as a previous step to reveal its etiology. We have screened 9 Peruvian families with markers of three different glaucoma locations in chromosomes 1 (1q24.3-q25.2), 2 (2qcen-q13) and 3 (3q21-q24). A family with 7 patients and 5 normal individuals, had dominant glaucoma cosegregating with markers from 2cen-q13, location of the glaucoma region (locus) GLC1B. This is the second study reporting association to markers associated to GLC1B. We have also seen that there is onset heterogeneity since there are adult and juvenile onset as reported originally before for other GLC1B families, with ocular pressures between 22 and 30 mm Hg. In our case, patients of the same generation had similar either adult or juvenile onset. One normal person elder than his affected brothers has a recombinant chromosome within the critical region: he shares markers cosegregating with glaucoma within the terminal side of the critical region whereas he does not share associated markers within the proximal side. We especulate that his normal status refines the location of GLC1B from 2qcen-q13 to 2qcen-q12. Acknowledgement: We thank the patients and their relatives by its participation. This work was supported by Universidad de San Martin de Porres Funds.

A QTL Influencing Triglyceride Levels Resides on Chromosome 13: The HERITAGE Family Study. *M.F. Feitosa¹, I.B. Borecki¹, T. Rankinen², J.P. Després³, A.S. Leon⁴, J.S. Skinner⁵, J.H. Wilmore⁶, M.A. Province¹, C. Bouchard², D.C. Rao¹.* 1) Div Biostatistics, Washington Univ, MO; 2) Pennington Biomedical Research Center, Louisiana Univ, LA; 3) Physical Activity Sciences Lab, Laval Univ, Canada; 4) Sch Kinesiology & Leisure Studies, Univ Minnesota, MN; 5) Dep Kinesiology, Indiana Univ, IN; 6) Dep Health & Kinesiology, Texas A & M Univ, TX.

Dyslipidemias are major etiologic contributors to atherosclerosis and coronary heart disease and targets for intervention. It is well known that lipids and lipoproteins are heritable and efforts to identify relevant quantitative-trait loci (QTL) are underway. Lipid profiles were assessed both at baseline and after 20 weeks of endurance-exercise training in 99 White and 101 Black families. Phenotypes were adjusted for age, sex, BMI, use of cardioprotective agents, hormonal intake and smoking; responses to training were further adjusted for baseline levels. A genomewide linkage scan involving 509 markers was conducted using a multipoint variance-components approach, separately by race and using race-specific marker allele frequencies. There was strong evidence for linkage on chromosome 13q12-14 with HDL triglycerides (TG) at baseline in White families, with the maximum multipoint lod score (LOD) of 3.9 ($p=0.00001$) at marker D13S1493. Suggestive linkage was also observed in the same region, at marker D13S219, with LOD of 2.2 ($p=0.00077$) for training response LDL-TG. Other suggestive linkages ($2.0 \leq \text{LOD} \leq 2.9$) were detected in White families on chromosomes 1q41-42 with LDL, 2p22-25 with LDL and 2p32 with TC/HDL, 5q22 with TC/HDL, 7p21 with LDL-APOB and LDL, 8q24 with LDL and LDL-APOB, 10-p14 with LDL-TG, 12q21-24 with HDL and HDL-TG, 14q31 with LDL-TG and TC, 15q26.2 with TC/HDL, 19p13 with LDL-TG, all phenotypes at baseline; and only 12q21-24 with HDL and HDL₂ in responses to training. In Black families, suggestive linkages were found on chromosomes 1q41-42 with TC and LDL-APOB at baseline, and 12q14 with TC/HDL in response to training. In summary, we have strong evidence for a QTL on 13q12-14 influencing triglycerides levels, both at baseline and in response to training.

Mapping a Gene for Dupuytren Contracture in Two Swedish Families. *F.Z. Hu^{1,2}, A. Nyström³, A. Ahmed¹, R. Dopico¹, I. Mossberg⁴, M. Palmquist⁵, J. Gladitz¹, M. Rayner⁶, J.C. Post¹, R.A. Preston¹, G.D. Ehrlich^{1,2}.* 1) Center for Genomic Sciences, Allegheny Singer Research Inst, Pittsburgh, PA; 2) Department of Human Genetics MCP Hahnemann School of Medicine, Pittsburgh, PA; 3) Departments of Plastic Surgery and Orthopedics, University of Nebraska School of Medicine, Omaha NB; 4) Department of Plastic Surgery, University of Umea, Umea Sweden; 5) University of Linkoping School of Medicine, Linkoping, Sweden; 6) Department of Otolaryngology University of Minnesota, School of Medicine, Minneapolis MN.

Dupuytren contracture (DC) (OMIM 126900) is widely regarded as the most common inherited connective tissue disease of mankind. It is characterized biochemically by increased levels of collagen III deposition and increased levels of hydroxylation and glycosylation of the collagen cross links. These phenomena are characteristic of granulomatous disease and scarification. Together with the fact that DC is more highly penetrant among persons with chronic hand trauma suggests that the DC phenotype results from a defect in the wound repair mechanism. DC is most frequently observed among the xanthocroi peoples of Northern Europe where its most common manifestations are thickening of the palmer fascia and contracture of the fingers. We have ascertained two large Swedish families in which Dupuytren contracture is inherited in an autosomal dominant manner with high penetrance by the sixth decade of life. In the current study blood was collected from all affected and informative unaffected family members for the performance of familial linkage analyses. Using a highly multiplex genotyping approach a genome-wide scan was performed at a resolution of 8 cM for all autosomes using the ABI PRISM linkage mapping set 2. Linkage was established to a 30 cm region of chromosome 16. Fine structure mapping of this region produced a maximal multipoint LOD score of 4.49 at marker D16S3068. We have identified a common allele at this marker for all affected persons in both families which is suggestive of a common founder.

Combined genome scan results for 266 pedigrees multiplex for systemic lupus erythematosus (SLE): significant evidence of genetic linkage at 1q22-23 and 11p13. *J.A. Kelly¹, J. Reid¹, J. Kilpatrick¹, B. Harris¹, C.K. Berry-Brattan¹, S. Frank¹, D. Hutchings¹, C. Cooney¹, S. Johnson¹, D. Bacino¹, K. Rossacci¹, T. Aberle¹, B. Namjou¹, S.K. Nath¹, G.R. Bruner¹, R.H. Scofield^{1,2,3}, J.A. James^{1,2,3}, J.B. Harley^{1,2,3}.* 1) Dept. Arthritis/Immunology, OMRF, OKC, OK; 2) Dept. of Medicine, University of Oklahoma Health Sciences Center, OKC, OK; 3) US Dept. of Veterans Affairs Medical Center, OKC, OK.

Systemic lupus erythematosus (SLE) is a complex genetic autoimmune disorder. We have previously reported results from an initial sample of 94 pedigrees with significant linkages at 1q22-23 and 1q41 identified in the 31 African-American pedigrees using model-based parametric methods. Using non-parametric methods and an additional 32 pedigrees, significant linkage was later reported at 4p16-15 in 77 European-American pedigrees. Here, we report results within the initial 126 pedigrees using model-based parametric methods and the results from an independent sample of 140 multiplex SLE pedigrees used to confirm previous findings as well as establish linkages in the combined 266 pedigrees. The first 126 pedigrees are linked at 1q22-23 (FcγRIIA, LOD=4.4), with eight regions demonstrating suggestive linkage (LOD³1.9) on chromosomes 1q, 3p, 7p, 11p, 11q, 12p, 12q and 13q. The additional 140 multiplex SLE pedigrees confirmed linkage to FcγRIIA (LOD=1.3), identified significant linkage at 11p13 (D11S1392, LOD=3.4) and four additional regions containing suggestive genetic linkages (1p, 3p, 7p and 14q). When the 266 pedigrees were combined, significant linkage was again identified at 1q22-23 (FcγRIIA, MLOD=3.9) and 11p13 (D11S1392, MLOD=3.8). Furthermore, a confirmatory effect at D16S3253, which, according to the UCSC December 2001 freeze, is < 1 cM telomeric to a significant linkage identified in Minnesota, was also observed (LOD=1.2), independently supporting linkage to this region. These data, together with other reported results, provide further evidence and independent confirmation of susceptibility genes for SLE residing at 1q22-23 and 11p13.

Linkage Analysis between Apo AI-CIII-AIV Cluster and Plasma Lipid Levels in Cardiovascular Disease Family.

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Background Cardiovascular disease (CVD) is a complex disorder, which the genetic contributions have significant roles with environmental factors. In the many studies, increased plasma lipid levels except HDL-cholesterol were directly correlated with cardiovascular risk. The aim of this study was to determine whether Apo AI-CIII-AIV gene cluster region (chromosome 11q23) contains specific loci that affect plasma lipid concentration or not. **Methods and Results** The 701 individuals from 93 CVD families were recruited. We collected genealogical informations for all participants and measured plasma lipid levels. Segregation analysis showed that the LDL-cholesterol follows the codominant Mendelian model with 61.2% of the variation and the equal transmission model. For HDL-cholesterol, the segregation analysis showed a dominant Mendelian model accounted for 51.8% of the variation. For linkage analysis, we selected 30 families (305 individuals) those have a relatively large number of subjects and multi-generation comparatively. In the analysis for the HDL-cholesterol, none of genetic markers at Apo AI-CIII-AIV gene cluster showed LOD score as evidence of linkage. In the analysis for the LDL-cholesterol, the highest LOD score was found at D11S912 of 130.9 cM (LOD score =1.18, p=0.099). To confirm the linkage between a putative gene that affects LDL-cholesterol level and D11S912, we reanalyzed the linkage with 21 families that favored a codominant Mendelian model, but there was no significant difference in LOD score. **Conclusion** The segregation analysis showed both LDL-cholesterol and HDL-cholesterol are under the genetic influences, following the Mendelian model. However, HDL-cholesterol showed no linkage to the Apo AI-CIII-AIV gene cluster, indicating the major gene effect influence in HDL-cholesterol is not explained by these loci. For LDL-cholesterol, we found the tentative linked loci at 11q23.

Is autosomal dominant nonsyndromic deafness in a Brazilian pedigree linked to DFNA7/DFNM1? *K. Lezirovitz, M.C.C. Braga, P.A. Otto, R.C. Mingroni-Netto.* Centro de Estudos do Genoma Humano, Dept Biol, Inst de Biociencias, Univ de Sao Paulo, Sao Paulo, Brazil.

Autosomal dominant inheritance accounts for 10% to 20% of hereditary nonsyndromic deafness. To date 34 nuclear *loci* associated with autosomal dominant nonsyndromic deafness have been described and among these 17 have been identified. We ascertained a large Brazilian family with 16 cases of autosomal dominant nonsyndromic sensorineural deafness. The majority of affected individuals have postlingual progressive hearing loss, initially affecting only high frequencies. The penetrance rate of the defect in this family was estimated to be 96% among individuals aged 35 years or more. Linkage analyses were performed in 24 individuals in four generations: seven of them had normal hearing, 16 were affected, and the affection status of one of them was unknown. The 35delG connexin 26 mutation and the A1555G mitochondrial mutation, two important causes of hereditary nonsyndromic deafness, were excluded. Linkage to DFNA2, DFNA10 and DFNA18, three *loci* involved in autosomal dominant nonsyndromic deafness, was also excluded. However, suggestive two-point lod scores were obtained within a region spanning ~20cM at 1q21-q24, which contains the DFNA7 *locus* as well as DFNM1, a modifier *locus* of DFNB26. The highest two-point lod score, 2.09 for $Q=0.07$, was obtained with marker D1S416. A 40 year-old individual who inherited the putative risk haplotype presents a slight hearing deficit (20dB) in all frequencies. If this individual is considered affected, the maximum lod score rises to 2.69 for this marker. However, a lod score of 5.76 would be expected in case of complete linkage. Nonpenetrant individuals and possible cases due to phenocopies could be influencing the results, but the possibility remains that more than one *locus* is involved in the manifestation of deafness in this pedigree.

Dark Dentate Disease (DDD - A Novel Dominantly-Inherited Spinocerebellar Ataxia Phenotype. *M.A. KNIGHT^{1, 2}, E. STOREY^{3, 4}, J. DIXON⁴, H. RUNDLE⁴, P.Q. THOMAS^{1, 2}, R.J.M. GARDNER^{1, 3}, S.M. FORREST^{2, 5}.* 1) MURDOCH CHILDRENS RESEARCH INSTITUTE, ROYAL CHILDRENS HOSPITAL, MELBOURNE, VIC; 2) DEPARTMENT OF PAEDIATRICS, UNIVERSITY OF MELBOURNE, MELBOURNE, VIC; 3) GENETIC HEALTH SERVICES VICTORIA, MELBOURNE, VIC; 4) THE DEPARTMENT OF NEUROSCIENCES, MONASH UNIVERSITY, ALFRED HOSPITAL CAMPUS, MELBOURNE, VIC; 5) THE AUSTRALIAN GENOME RESEARCH FACILITY, WEHI, PARKVILLE, VIC.

SCAs 1, 2, 3, 6 or 7 account for a minority of Australian pedigrees with dominant spinocerebellar ataxia (SCA). This prompted a search for SCA pedigrees with novel phenotypes, resulting in this description of DDD. We have studied a large Anglo-Celtic kindred with thirteen affected members. A notable observation has been the calcification of the dentate nucleus, palatal tremor and dysphonia in family members. The age of onset ranged from 19-64 years. Pedigree information does not permit a firm conclusion as to whether or not anticipation exists. Gene testing excluded the known SCA loci for SCAs 1, 2, 3, 6, 7 and 8, and molecular linkage analysis excluded SCAs 4, 5, 6, and SCAs10-17. Thus we hypothesised that this might represent a new SCA clinical phenotype necessitating the performance of a full genome scan. Simulation analysis of the pedigree indicated a maximum LOD score of 6.9 with the power to exclude at an average of 10.6-cM either side of any marker. Upon analysis of the genome-wide scan we found linkage to chromosome 11, very close to the existing locus for SCA5. While DDD maps to the same broad region of chromosome 11 as SCA5, it is phenotypically quite distinct from that disorder, in which dentate calcification, palatal tremor and dysphonia have not been seen in the two pedigrees reported to date. This suggests that we may have a novel phenotypic variant of SCA5 or a new locus that maps in the vicinity of SCA5 on chromosome 11. Subsequent fine mapping in this area has revealed that linkage to the SCA5 locus cannot be excluded, and further collaborative work to define the marker order in this region is in progress. Candidate genes identified using bioinformatics are currently being screened to identify the disease-causing mutation.

Identification of loci involved in familial idiopathic scoliosis. *C.M. Justice*¹, *N.H. Miller*², *B. Marosy*², *J. Zhang*³, *A.F. Wilson*⁴. 1) Genometrics Section, NHGRI/NIH, Baltimore, MD; 2) Department of Orthopaedic Surgery, Johns Hopkins University, Baltimore, MD; 3) Department of Genetics, Johns Hopkins School of Medicine, Baltimore, MD; 4) CIDR, Johns Hopkins University, Baltimore, MD.

Idiopathic scoliosis (IS) is a structural lateral curvature of the spine present in the late juvenile or adolescent period in otherwise normal individuals. It affects 2-3% of the pediatric population, and 0.2-0.5% of the population require active treatment. Idiopathic scoliosis is believed to be a complex genetic disorder in which expression of the disease state may depend on several genetic and possibly environmental factors. Previous studies have suggested autosomal dominant, X-linked and multifactorial modes of inheritance. In this study, 204 families with at least two individuals with a lateral curvature greater than or equal to 10 degrees were ascertained and clinically characterized. Phenotypes include degree of lateral curvature, curve type, age of onset and sex. A genome-wide screen for 1198 individuals was performed at the Center for Inherited Disease Research, using a modified Weber marker set consisting of 391 short tandem repeat markers. In order to reduce heterogeneity and maximize the possibility of identifying potential loci for the disease trait, scoliosis was analyzed both as a quantitative trait and as a qualitative trait, in which the curvature determining the threshold for affectation status was set at values of 10, 20, 30, 40 and 50 degrees. The families were also stratified on the ratio of the likelihood of each family given an X-linked model relative to that of an autosomal model. Model independent sib-pair linkage analysis was performed on these various subsets. Candidate regions were identified on chromosomes 1, 6, 8, 9 and 16.

A novel susceptibility gene for systemic lupus erythematosus (SLE), SLER1, maps to chromosome 5p15.3 in multiplex SLE families overlapped with rheumatoid arthritis. *S.K. Nath, B. Namjou, J. Kilpatrick, J.A. Kelly, J.A. James, J.B. Harley.* Arthritis & Immunology, Oklahoma Medical Res Fndn, Oklahoma City, OK.

Systemic lupus erythematosus (SLE) is a chronic, complex and genetically heterogeneous human autoimmune disease. For disorders with a poorly known biochemical basis, like SLE, identification of the genes is a prerequisite or key to their understanding its biological basis. Despite considerable effort to identify susceptibility loci for SLE, no specific genes have yet been localized by human SLE genome scan approach. SLE is often associated with other autoimmune diseases such as rheumatoid arthritis (RA), which may indicate a common genetic predisposition for both of these disease manifestations. We identified 36 families multiplex for both SLE and RA: 19 African-American (AA), 14 European-American (EA) and 3 others from our ongoing SLE genetics project. Genome-wide linkage analyses were conducted using 330 microsatellite markers separately for each ethnic group. Based on the initial genome scan, a significant evidence of linkage (LOD=5.3) was identified at chromosomal location 5p13.3 for EA families and a suggestive evidence (LOD=2.45) of linkage was identified at chromosomal location 4q for AA. Conversely, on chromosome 5 the maximum lod score for AA was .77, and on chromosome 4 the maximum for EA was .20. Then 6 additional markers were genotyped on 5p15.3 for further narrow the linkage interval. The maximum parametric and non-parametric LOD scores were 6.9 and 6.2, respectively. The P-values associated with these lod scores were 1.7×10^{-8} and 2.6×10^{-7} , respectively. The linked region spans about 16 cM. To our knowledge this is the most significant evidence of linkage for SLE to date. These results suggest that grouping families based on their clinical features and racial origin not only isolates genetically homogeneous families but also increases power to detect linkage for complex diseases like SLE.

Autosomal Dominant Stargardt Macular Dystrophy: Clinical Features and Linkage Analysis in A Large Greek Family.

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Purpose: Stargardt macular dystrophy (STGD) is the most common hereditary macular dystrophy and is characterized by decreased central vision, macular atrophy and flecks. In most instances STGD is inherited as an autosomal recessive or simplex trait. A recessive locus was assigned to chromosome 1p (STGD1). Loci for autosomal dominant Stargardt-like macular dystrophy have been mapped to chromosomes 6q (STGD3) and 4p (STGD4). We describe our effort to characterize clinical phenotype and map the disease gene in a large Greek family. **Methods:** Clinical examinations were performed. Genomic DNA was obtained from the blood of 29 members and genotyped using STR markers. Linkage analysis was performed using the LINKAGE 5.1. **Results:** We describe a large five-generation pedigree with dominant Stargardt-like macular dystrophy from Epirus, Greece. The family is consisted of 234 individuals with 24 affected members. Clinical examination revealed visual acuity ranging from 20/25 to 20/200 with age at onset between 7 and 18 years. Fundoscopic examination showed loss of foveal reflex, "beaten bronze" appearance of the foveal region, and flecks of varying degrees. The disease was symmetric in the two eyes and progressive with age. Linkage analysis with STR markers excluded linkage to known STGD loci, and a genome wide search is in progress. **Conclusions:** We have identified a family with Stargardt-like macular dystrophy likely representing a new disease locus. Identification of another causative gene for STGD will aid in our understanding of the pathogenesis of this disease, and allow the development of therapeutic agents.

Linkage analysis of primary microcephaly in Indian families. A. Kumar¹, S. Blanton², M. Markandaya¹, S.C. Girimaji³. 1) MRDG, Indian Institute of Science, Bangalore, India; 2) Department of Pediatrics, University of Virginia Health Science Center, Charlottesville, USA; 3) Department of Psychiatry, NIMHANS, Bangalore, India.

Microcephaly (small head) is a condition in which the head circumference of an affected individual is greater than 3 SD below the mean for age and craniosynostosis is not present. Microcephaly occurs due to a variety of reasons such as intrauterine infections, drugs taken during pregnancy, prenatal radiation exposure, maternal phenylketonuria and birth asphyxia, which are the rare causes of microcephaly. The majority of microcephaly cases arise as the result of a variety of genetic mechanisms including cytogenetic abnormalities, single-gene disorders and syndromes of as-yet-undetermined etiology. Primary microcephaly (MCPH, MIM 251200) appears to be a distinct subtype. It is defined by the absence of associated malformations and of secondary or environmental causes. It is inherited as an autosomal recessive trait. Mental retardation in primary microcephaly ranges from mild to severe, but other neurological deficits are absent. Primary microcephaly shows genetic heterogeneity with five known loci: MCPH1 on chromosome 8p22-pter, MCPH2 on chromosome 19q13.1, MCPH3 on chromosome 9q34, MCPH4 on chromosome 15q and MCPH5 on chromosome 1q31. We have ascertained nine families with primary microcephaly from India. On examination, the head circumferences of the affected individuals were found to be greater than 5-7 SD below the population age-related mean. All the affected individuals had mild to severe mental retardation and none were able to read and write, but could speak at least a few words and had basic self-care skills. To establish linkage of these nine Indian families to known MCPH loci, microsatellite markers were selected from the candidate regions of each of the five known MCPH loci and used to genotype the families. The results showed that 3/9 families were linked to the known MCPH5 locus on chromosome 1q31 with a combined lod score of 5.3864. The work is in progress to establish linkage of other six families using a genome-wide linkage screen (Supported by SDTC grant PC11014).

Genetic analysis of spirometric measurements. *A. Malhotra¹, R.E Kanner², M.F. Leppert¹, S.J. Hasstedt¹.* 1) Department of Human Genetics, University of Utah, Salt Lake City, UT; 2) Pulmonary Division, University of Utah, Salt Lake City, UT.

Lung function defects such as chronic obstructive pulmonary disease (COPD) are a common and increasing cause of death worldwide. Although environmental factors such as cigarette smoking are associated with COPD, only 10-20% of heavy cigarette smokers develop this disease. Therefore, genetic factors may play a role in the etiology of COPD. In addition, familial clustering and heritability estimation studies of traits related to lung function suggest the role of genes.

FEV₁ (Forced Expiratory Volume in 1 second) and FVC (Forced Vital Capacity) were measured on 264 members of 26 Utah pedigrees. These volume measurements are reduced in lung function defects and serve as criteria in the diagnosis of certain lung function abnormalities. In addition, a reduced FEV₁/FVC ratio is observed in individuals suffering from airways obstructions. Genetic analysis of these phenotypes will provide further insight into the role of genetic factors in the development of lung disease.

A segregation analysis of the FEV₁/FVC ratio has led to the inference of a major locus underlying the inheritance of this phenotype. Assuming the inferred dominant and recessive models, two-point and multipoint linkage analyses were performed using GENEHUNTER. Two-point lod scores of >2 (highest lod score = 2.70) were observed on chromosome 5q. A multipoint lod score of >3 was obtained in this region providing evidence for linkage. This region will be fine mapped to further localize the gene. No model could be inferred for the traits FEV₁ and FVC. Nonparametric linkage analyses were performed on FEV₁, FVC, and the ratio FEV₁/FVC. Two-point lod scores of >1.5 were obtained in several regions suggesting the presence of genetic heterogeneity. Multipoint linkage analyses are currently being performed for all three phenotypes.

Search for linkage disequilibrium with febrile seizures in the *FEB4* region on chromosome 5. J. Nakayama^{1, 2}, N. Yamamoto¹, K. Hamano³, N. Iwasaki², A. Matsui², T. Arinami¹. 1) Department of Medical Genetics, Institute of Basic Medical Sciences, University of Tsukuba, Ibaraki, Japan; 2) Department of Pediatrics, Institute of Clinical Medicine, University of Tsukuba, Ibaraki, Japan; 3) Department of Pediatrics, Kitaibaraki Municipal General Hospital, Ibaraki, Japan.

Febrile seizures (FS) are the most common form of childhood seizures, and they occur in about 7% of Japanese children. Three putative FS loci, *FEB1* (chromosome 8q13-q21), *FEB2* (chromosome 19p), and *FEB3* (chromosome 2q23-q24) linked to some large Caucasian families have been mapped. Furthermore, we have previously reported significant evidence for linkage of febrile seizures to chromosome 5q14-q15 (*FEB4*) in Japanese families (Hum Mol Genet 2000;9:87-91). Significant linkage disequilibrium between FS and the marker D5S652 was observed. To identify responsible mutation(s) for FS, we screened three genes, the proprotein convertase subtilisin/kexin type 1 gene (*PCSK1*), the calpastatin gene (*CAST*), and the type 1 tumor necrosis factor receptor shedding aminopeptidase regulator gene (*ARTS-1*), which have been mapped within 500kb from this marker. The entire coding region of these genes was screened by single-strand conformation polymorphism (SSCP) or denaturant high-performance liquid chromatograms (DHPLC) analysis in 48 unrelated Japanese patients with FS. A total of 61 polymorphisms/variants were identified. We genotyped these polymorphisms by PCR-RFLP and direct sequencing methods in 48 FS families (n=192) and performed the transmission disequilibrium test. The IVS21-65A allele of *CAST* and the -103T allele of *PCSK1* were more frequently transmitted to FS patients (p=0.0095 and p=0.032, respectively). We also identified 21 polymorphisms/variants within 5kb from D5S652. We examined 4 polymorphisms of these polymorphisms by TDT and significant linkage disequilibria between these polymorphisms and FS were observed. Our data indicate that there is a gene responsible for FS between *PCSK1* and *CAST*, probably near D5S652, although no known gene has been mapped around D5S652.

Refinement of the candidate region for spinocerebellar ataxia type 4 (SCA4) in a large German kindred. *Y. Hellenbroich, C. Zuehlke, H. Pawlack, E. Schwinger.* Institute of Human Genetics, University of Luebeck, Luebeck, Germany.

Spinocerebellar ataxia type 4 (SCA4) is an autosomal dominant disorder mapped to chromosome 16q22.1 in a large Utah kindred. The clinical phenotype is characterized by cerebellar ataxia with sensory neuropathy. In addition, a pure cerebellar ataxia in six Japanese families is also linked to the same candidate region suggesting to be an allelic disorder with different clinical features. Here, we present a five-generation family from northern Germany with clinical findings similar to the SCA4-family from Utah linked to the same locus. A maximum lod score of 5.21 ($q=0.00$) was achieved with marker D16S496 in a genomewide linkage scan with a panel of 402 markers. Haplotype analyses further refined the gene locus in a 3.69-cM interval between D16S3019 and D16S512. Furthermore, we investigated the interrupted CAG sequence of the HRIHFB2206 gene coding for the largest polyglutamine tract of all known genes in the candidate region and could exclude a repeat expansion at this locus. Nevertheless, the anticipation of disease onset and severity in this family points to a repeat expansion as the underlying mutation for SCA4.

A genome-wide scan identifies a major susceptibility locus for leprosy susceptibility in Vietnamese families. *M.T. Mira*¹, *A. Alcaïs*², *N.V. Thuc*⁴, *N.T. Huong*⁴, *V.H. Thai*⁴, *M. Girard*¹, *A. Verner*³, *M.C. Phuong*⁴, *T.J. Hudson*³, *L. Abel*², *E. Schurr*¹. 1) McGill Centre for the Study of Host Resistance, McGill University, Montreal, Canada; 2) INSERM U 436, Paris, France; 3) Montreal Genome Center, Montreal, Canada; 4) Dermatology Hospital, Ho Chi Minh City, Vietnam.

Leprosy, an infectious disease caused by *Mycobacterium leprae*, presents itself in different clinical manifestations, ranging from single lesion paucibacillary to multiple lesion multibacillary forms. To identify genomic regions linked with susceptibility to leprosy, we performed a genome-wide scan in leprosy families from South Vietnam containing both paucibacillary and multibacillary cases. A total of 87 multiplex nuclear families (433 individuals) were genotyped for 395 highly informative microsatellite markers spanning the entire human genome and interspersed by 10 cM on average. Non-parametric linkage analysis initially pointed to 11 genomic regions distributed along 9 chromosomes showing suggestive evidence for linkage with either leprosy per se or leprosy type (lod score > 1.5). The addition of a large number of new microsatellite markers brought the information content along all the 11 regions to 95% on average. Analysis of the high density maps revealed strong evidence for linkage between one chromosomal region and leprosy "per se" (lod score of 4.21; $P = 0.000005$). Furthermore, association between leprosy and microsatellite markers underlying the peak lod score was detected in a family-based study using an independent sample of 208 simplex Vietnamese families ($P = 0.00006$). Finally, chromosomal region 6p21, overlapping the HLA complex, presented a lod score of 2.85 ($P = 0.00016$) when tested for leprosy "per se", and 2.64 ($P = 0.0002$) when tested for leprosy type. The remaining 9 regions submitted to fine mapping failed to show significant evidence for linkage with the tested phenotypes (lod scores < 1.5). These results strongly suggest the existence of at least one major locus for the control of susceptibility to leprosy "per se". They are also in accordance with the generally accepted idea for a role of the HLA region in controlling disease susceptibility and clinical form of leprosy.

Confirmation of linkage and refinement of a new gene locus for Nephronophthisis type 4 and Senior-Loken syndrome on chromosome 1p36.3. *E. Otto¹, J. Hoefele¹, R. Ruf¹, A.M. Mueller¹, K.L. Hiller¹, M.T.F. Wolf¹, M.J. Schuermann¹, A. Becker¹, H.C. Hennies², P. Nuernberg², F. Hildebrandt¹.* 1) Department of Pediatrics, University of Michigan, Ann Arbor, MI; 2) Mikrosatellitenzentrum, MDC, Berlin, Germany.

Nephronophthisis (NPH), the primary genetic cause for chronic renal failure in children, is an autosomal recessive cystic kidney disease. The association of NPH with retinitis pigmentosa is known as Senior-Loken syndrome (SLS). Three gene loci (NPHP1, 2, and 3) have been mapped for NPH, while only the gene (NPHP1) for NPH1 has been identified. Some SLS patients also show linkage to the NPHP1 and NPHP3 loci. By total genome search for linkage we recently identified a new gene locus (NPHP4) for NPH type 4 and SLS in 6 NPH multiplex families and in one large SLS kindred (*Am J Hum Genet* 70:1240, 2002).

Following exclusion of mutations in the NPHP1 gene and of linkage to NPHP2 and NPHP3, we here performed haplotype analysis at the NPHP4 locus in 38 additional multiplex families with NPH (24 families) and with SLS (14 families). Haplotypes demonstrated compatibility with linkage to NPHP4 in 14 out of 24 NPH families (58%) and in 9 out of 14 SLS families (64%).

Furthermore, newly generated polymorphic markers were typed on key recombinants from F30, narrowing the disease locus to 1.2 Mb in size.

We conclude that linkage to a fourth locus (NPHP4) for NPH and SLS was confirmed, and that there is evidence for at least one additional locus for NPH and SLS. Mutational analysis of candidate genes is now performed to identify the gene responsible for NPH type 4 and Senior Loken Syndrome.

A novel locus for non-syndromic autosomal dominant hearing loss mapped to chromosome 16p13.3. *X.C. Li¹, S.A. Kim¹, H.M. Saal², R.A. Friedman¹*. 1) Dept Cell & Molecular Biol, House Ear Inst, Los Angeles, CA; 2) Human Genetics Division, Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH.

Hearing loss is the most common sensory defect in humans. A genetic linkage study was conducted on a large multigenerational US family with nonsyndromic autosomal dominant progressive hearing loss. The age of onset of the hearing impairment in this family varies from the first decade to the early twenties. Most affected individuals have demonstrated bilateral severe-to-profound sensorineural hearing loss, mainly in the middle and high frequencies. The deafness gene segregating in this family is mapped to a 10 cM interval near the telomere region on chromosome 16p13.3. A maximum lod score of 4.07 was obtained with marker D16S3401 at $q = 0$. Several good candidate genes have been identified in this interval, such as a voltage-dependent calcium channel gene *CACNA1H*, a chloride channel gene *CLCN7* and two claudin genes.

Evidence suggesting BRD2 is the EJM1 locus for Juvenile Myoclonic Epilepsy. *D.K. Pal¹, O. Evgrafov³, M. Durner², C. Gilliam³, D.A. Greenberg².* 1) Psychiatry, Columbia University, New York, NY; 2) Stat Genet Divn, Biostatistics Dept, Columbia University, New York, NY; 3) Genome Center, Columbia University, New York, NY.

Several reports have demonstrated linkage of juvenile myoclonic epilepsy to the HLA region of chromosome 6. Our group found significant association to alleles at the DQB1 and BRD2 loci (1). The objectives of the present study were to define the region more precisely using densely spaced SNP markers and to identify possible causative mutations. **METHODS:** 20 JME probands, taken from families with positive lod scores (lod >0.1) at the 6p locus, and 46 unlinked JME probands, were typed for 11 SNPs between HLA-DP and HLA-DQ. Haplotype frequencies were compared in a case-control design. Additionally, all exons and splice sites in the region were tested for mutations in four linked families. **RESULTS:** One consistent haplotype was identified in positive lod score families, which was significantly more frequent than in comparison chromosomes (Odds ratio: 5.8, 95% CI:1.7-19.4, p=0.001). This haplotype extended to the boundary of the BRD2 gene and contained the associated CA repeat allele. There was no significant evidence of linkage disequilibrium beyond the gene boundaries. Sequencing of 20 probands from families with positive lod scores revealed 3 missense mutations, 1 small deletion and 1 DNA alteration which could potentially affect splicing. These variants, and 2 other missense mutations described in the literature, were not consistently associated with JME. **CONCLUSION:** Other reports of loci in common disease also have failed to find consistent mutations in exons of associated loci. While we cannot yet exclude the rest of the DQ-DP HLA region, the presence of a SNP haplotype, plus our evidence of association with an allele of a microsatellite marker only in linked families, continues to suggest that BRD2 is EJM1. 1. Greenberg DA, Durner M, Keddache M, et al. Reproducibility and complications in gene searches: linkage on chromosome 6, heterogeneity, association, and maternal inheritance in JME. *Am J Hum Genet.* 2000 Feb;66(2):508-16. Supported by grants: NS31775, NS27941 and MH48858.

Fine mapping of a schizophrenia susceptibility locus on 13q32 in a Canadian Sample. *D.M. Waterworth¹, E.W.C. Chow², A.S. Bassett², L.M. Brzustowicz^{1,3}*. 1) Dept Genetics, Rutgers Univ, Piscataway, NJ; 2) Department of Psychiatry, University of Toronto, and Schizophrenia Research Program, Queen Street Division, Centre for Addiction and Mental Health, Toronto, Ontario; 3) Department of Psychiatry, UMDNJ, New Jersey Medical School, Newark.

Schizophrenia is a complex and variable disorder that is characterized by cognitive, social and affective impairment, and by the presence of psychotic symptoms. In our previously reported genome scan of 22 Canadian families (n=297), an HLOD of 3.81 at D13S793 on 13q32-33 was found, replicating previous findings at D13S174 (around 9cM proximal to D13S793) by Blouin et al in multiplex pedigrees of mostly European descent (NPL 4.18; p= 0.00002). Bipolar disorder has also been linked to this region in a number of studies. Since our last report, a number of additional subjects with DSM-III-R diagnoses and two additional families have augmented the size and power of the collection (n=350), though a small number of subjects currently have a provisional diagnosis. A multipoint using the same markers as previously reported generated an HLOD of 2.52 at D13S793 in this extended data set. A dense map (average 0.8 cM spacing) of microsatellite markers was constructed around D13S793 on 13q32 in order to refine the position of this putative locus. Eighteen additional microsatellite markers were typed over a 16cM (»13Mb) region, ranging from D13S1300 to D13S274. Parametric linkage analysis was performed using LINKAGE for four models, dominant or recessive for each of a narrow or broad diagnostic classification, as before. The maximum 2-point LOD score was obtained for D13S154 (HLOD 2.7) with the broad dominant model. One large family generates a LOD score of 2.3 alone, and restricts the linked interval to between D13S71 and D13S786. Although the addition of new markers and subjects has led to reduction in the LOD score at this locus, the families that appear linked are consistent in terms of location and model. The close proximity between our linkage peak and that of the bipolar groups suggests that the two disorders may indeed share a common susceptibility locus on 13q32.

Canine model of idiopathic epilepsy. *M.C. Roberts¹, E.E. Patterson², P.J. Armstrong², J.R. Mickelson¹*. 1) Veterinary PathoBiology, Univ Minnesota, St Paul, MN; 2) Small Animal Clinical Sciences, Univ Minnesota, St Paul, MN.

Mutations in a number of genes for primary idiopathic epilepsy (IE) have recently been defined in humans, however there are many more forms of IE for which the mode of inheritance is complex and the genetic basis has not been identified. The domestic dog has a high occurrence of IE, with up to 3% of purebred dogs being affected. Because of a strong founder effect, purebred lines of dogs can be very useful for dissecting the genetic basis of the more complex human conditions. The Vizsla is a breed in which we have identified lines of dogs that produce pups with IE. Around three years of age the affected dogs suffer from partial seizures, partial onset seizures with secondary generalization, and/or primary generalized seizures with a median frequency of nine a year. DNA and phenotypes have been collected from 159 dogs that can be assembled into one large pedigree or nine smaller pedigrees that span three generations. Segregation analysis suggests an autosomal recessive mode of inheritance, but a polygenic model with a gene of major effect cannot be ruled out. A full genome scan has been initiated on these dogs, utilizing 295 polymorphic canine microsatellite markers with an average spacing of 11 cM. There are seven gaps greater than 20 cM and two of the smaller chromosomes have only one informative marker. Parametric and nonparametric linkage analysis has identified nine possible regions of interest. Follow-up marker analysis will be done on these regions and other areas where the markers are not highly informative in the pedigrees. Identification of genes for epilepsy in this and other breeds of dog will be very useful for comparative genetic studies of human epilepsy.

Linkage analysis of quantitative traits related to Syndrome X and stature on Kosrae, Federated States of Micronesia. *D. Shmulewitz¹, S.C. Heath², Z. Han¹, L. Petukhova¹, S.A. Auerbach³, M.L. Blundell¹, S. Boger¹, S. Oplanich¹, S. Signorini¹, P.C. Verlander¹, J.D. Winick¹, M. Yifrach¹, J. Ott¹, J.L. Breslow¹, M. Stoffel¹, J.M. Friedman¹.* 1) Starr Center for Human Genetics, The Rockefeller University, New York, NY; 2) Centre National de Genotypage, Evry, France; 3) Health Resources and Services Admin., Dept. of Health and Human Services, New York, NY.

Syndrome X, a term used for the observed clustering of metabolic disorders, includes obesity, type II diabetes, hypertension, and dyslipidemia/heart disease. These disorders have been shown to have underlying complex genetic inheritance. We performed a complete genome scan for height and 14 quantitative traits related to these disorders in the population on the Pacific island of Kosrae, which has a high prevalence of the Syndrome X disorders. The present study included 1709 individuals from Kosrae who are part of one large pedigree. These have been genotyped for 360 microsatellite markers (10.2 cM autosomal map) using the ABI 3700 capillary electrophoresis methods at the Genotyping Core Facility at Rockefeller University. Linkage analysis was carried out with the reversible jump Markov chain Monte Carlo method Loki, which can carry out segregation and linkage analysis on quantitative traits in large pedigrees with multipoint analysis and covariate corrections (Heath, 1997). This resulted in the identification of quantitative trait loci for body mass index (chromosome 18), serum leptin levels (5), hip circumference (10), weight (18), fasting blood sugar (1), systolic blood pressure (20), arterial blood pressure (9), apolipoprotein B (2), total cholesterol (12, 19), and height (1, 5, 10, 15, 19). These are promising regions that merit further study, to try to identify the causal genes for these traits and their effect on disease pathogenesis. Heath, S. (1997). Markov chain Monte Carlo segregation and linkage analysis for oligogenic models. *Am.J. Human Genet.* 61: 748-60.

Genome wide screen of a panel of multigenerational families in Adams Oliver Syndrome. *A. Skoura¹, S. Sifakis¹, F. Goodman², R. Winter², A. Lin³, D. Basel¹, P. Beighton⁴, M.W. Kilpatrick¹, P. Tsipouras¹.* 1) Pediatrics, UConn Health Center, Farmington, CT; 2) Molecular Medicine, ICH, London; 3) Dept of Newborn Med, Childrens Hosp, Boston MA; 4) Human Genetics, UCT, Cape Town.

The Adams Oliver syndrome is a heterogeneous disorder characterized by aplasia cutis congenita of the scalp, truncation defects of the distal limbs and a myriad of associated clinical presentations which include cardiac anomalies, cutis marmorata, renal anomalies, polymicrogyria and a variety of other neurological manifestations. The observed phenotypic variability in this disorder complicates the clinical diagnosis and the identification of a causative gene would be of great diagnostic benefit. In addition, the identification of the genetic basis of this phenotype has the potential to further our understanding of the intricate developmental pathways that direct the embryonic ectoderm. To this end, we have undertaken a genome wide scan using a human marker screening set, which comprises 378 autonomic primer pairs that cover the entire genome at an average spacing of 9cM. The initial phase of the screening was limited to the single multigenerational family available to us at the time, which comprised 14 individuals, 6 of which were phase known meioses. Two-point lod scores were calculated using Mlink and considered significant if greater than 2. Using this criterion, several loci were found to be significant, generating 2-point lod scores of 2.24 (D2S1790, 2p11.2), 2.17 (D13S1807, 13q14) and 2.21 (D15S1507, 15q22). Additional markers were then used to further delineate these regions and the next phase of the project was initiated. This second phase included expanding the cohort to include 4 additional families, bringing the total number of individuals investigated to 48. The total number of affected persons now amounts to 23, of which 15 are phase known meioses. This expanded panel of five families is currently being analysed in an attempt to identify the locus responsible for this disorder. In addition, we will utilize this data to investigate the possibility of genetic heterogeneity, as has been observed for several other skeletal dysplasias.

A validated set of SNPs for linkage mapping studies. *M.D. Rhodes¹, D. Dailey¹, P. Dong¹, D.A. Gilbert¹, C. Scafe¹, Y. Wang¹, M. Laig-Webster¹, X. Su¹, R. Koehler¹, H. Avi-Itzhak¹, J. Ziegler¹, L. Wogan¹, E. Spier¹, F. de la Vega¹, E. Reied², J. Brooking³, F. Dudbridge³, A.M. Dearlove³, M.R. Yuille³.* 1) Genomic Assays Business, Applied Biosystems, Foster City, CA; 2) Department of Medical Genetics, University of Cambridge, Cambridge CB2 2XY UK; 3) MRC geneservice (UK HGMP RC), Babraham, Cambridge CB2 4AT UK.

We will present data on a validated set of SNPs selected to be used for human linkage mapping experiments. These SNPs were selected by the SNP consortium (<http://snp.cshl.org>) as a subset of all the SNPs analyzed. The SNPs were chosen based their positions and informativeness based on initial population frequency .In collaboration with the SNP consortium Applied Biosystems and Celera Genomics then undertook further validation studies.

The first step of this process was to design 5 nuclease assays using Taqman probes for all 2000 identified assays . Population frequencies for these assays were assessed in three different populations, African-American, Asian and Caucasian. The markers were also typed against several multi-generational CEPH families to ensure mendelian inheritance and generate genetic distances.

Data will be presented on the distribution of the SNPs across the genome, in genetic and physical distances, comparisons with existing Microsatellite mapping sets will be shown. Practical use of the SNP set will be presented based on a retrospective study of hereditary spastic paraplegia . . The practical and analytical Implications of shifting from microsatellites to SNPs will be discussed.

Contribution of genetic variants at CARD15 and the chromosome 5q31 locus to Crohn's disease. *C.G. Mathew¹, M. Mirza¹, S. Fisher¹, K. King¹, J. Hampe², J. Sanderson³, A. Forbes⁴, J. Mansfield⁵, A. Cuthbert¹, S. Schreiber², C.M. Lewis¹.* 1) Div Med & Molec Genetics, GKT Sch Medicine, King's Coll London, Guy's Hospital, London, UK; 2) Dept General Internal Medicine, Christian-Albrechts-University, Kiel, Germany; 3) Dept Medicine, GKT Sch Medicine, King's Coll London, St Thomas' Hospital, London, UK; 4) St Mark's Hospital, Harrow, Middlesex, UK; 5) Dept Gastroenterol Hepatol, Univ Newcastle, Royal Victoria Infirmary, Newcastle,.

Inflammatory bowel disease (IBD) is a complex disorder with 2 phenotypic classes, Crohn's disease (CD) and ulcerative colitis (UC). Rioux et al (Nat Genet 29:223-8) recently reported the association of a common haplotype in the cytokine gene cluster on chromosome 5q31 with CD. We have tested this locus for association with disease in a large cohort of European families and patients with IBD. A single nucleotide polymorphism representative of this haplotype (SNP IGR2063b) was genotyped in families containing a total of 511 CD and 320 UC offspring, and tested for association by transmission disequilibrium. Excess transmission of the G allele of C2063G was observed in CD ($p=0.011$), but not in UC. The SNP was then genotyped in 414 unrelated British cases of CD, 387 cases of UC and 362 controls. The G allele frequency was 50.2% in CD, 40.1% in UC and 42.4% in controls, thus confirming significant association with CD ($p=0.0024$). The combined effect of the 5q31 and CARD15 loci was investigated in 558 unrelated CD patients who had been genotyped for the C2063G SNP and for the 3 disease susceptibility SNPs in CARD15. The frequency of SNP 2063G in CD patients with CARD15 genotypes of $-/-$, $-/+$ and $+/+$ was 45.0%, 54.4% and 56.6% respectively. Logistic regression was used to estimate risks conferred by the 5q31 and CARD15 loci. The risks for the 2063CG and GG genotypes in the absence of CARD15 mutations were 1.23 and 1.52 respectively. The highest risk for CD was 32.68 for the genotype CARD15 $+/+$, 5q31 2063GG. The combined population attributable risk for these 2 loci was 62%. Our data therefore provide support for the existence of a CD susceptibility gene of moderate effect on chromosome 5q31.

An Insertion/Deletion Polymorphism in the Human Immunoglobulin V_H Region Identified by Analyzing Single Spermatozoa with 32 Markers. *S. Pramanik, H. Li.* Department of Molecular Genetics, Microbiology and Immunology/The Cancer Institute of New Jersey, UMDNJ-Robert Wood Johnson Medical School, 195 Little Albany Street, New Brunswick, NJ 08903, USA.

The presence of relatively large insertion/deletion polymorphisms in the human immunoglobulin V_H region is implicated by many studies. However, the extent, location and impact of these polymorphisms remain unclear because these polymorphisms are mostly present in heterozygous state, human somatic cells are diploid and it is difficult to determine the physical structure of a region on single chromosomes. The present study was designed, for the first time, to identify insertion/deletion polymorphisms by analyzing a large panel of selected marker sequences in single sperm. Thirty-two markers were selected as unique sequences spaced by ~5 kb that were located near the 3' end of the V_H region. A two-round multiplex PCR protocol was used to amplify these marker sequences from single sperm samples prepared from nine unrelated healthy sperm donors. The parental haplotypes with respect to these markers in the sperm donors were determined by examining the presence or absence of these markers. Results indicate that seven clustered markers in six of the 18 haplotypes were missing and likely represented an insertion/deletion polymorphism with a size of ~35 kb. The ratio for the sperm donors homozygous for the insertion haplotype, the heterozygous haplotype and the homozygous for the deletion haplotype was 4:4:1 which perfectly matched the expectation from Hardy-Weinberg Equilibrium. Three V_H gene segments (2-10P, 3-9 and 1-8), of which two are functional are affected by this insertion/deletion polymorphism. Our results indicate that >10% individuals in the human population may not have these gene segments in their genome and ~ 44% may not have these gene segments in one of their two haplotypes. The biological impact of this polymorphism would be very interesting to study. The approach used in this study could be applied to understand the physical structure and diversity in many chromosomal regions.

Mutation screening of the Split Hand Foot Malformation (SHFM3) by Positional Transcript Analysis. *D. Basel*¹, *T. Homfrey*², *M.W. Kilpatrick*¹, *P. Tsipouras*¹. 1) Pediatrics, Univ Connecticut Hlth Ctr, Farmington, CT; 2) St. Georges Hospital Medical School, London, UK.

Split Hand-Foot Malformation (SHFM) is a non-syndromic disorder, characterized by variable defects of the hands and feet. It is known to occur in a sporadic or familial fashion and has an incidence of 1:18,000 livebirths. SHFM3 was localized to 10q24 in 1996 and it was anticipated that the human homologue of the mouse gene for *Dactylaplasia*, a murine mutant closely resembling the SHFM3 phenotype, would harbor a causative mutation. The absence of a mutation, either in this gene, or in several candidate genes, including FGF8, neuralized, beta-transducin, CHUK, WNT8b and SFRP5, selected for their potential role in the embryonic development of the apical ectodermal ridge, led us to develop a new approach that we term Positional Transcript Analysis (PTA). We first defined a narrow critical region, spanning 4 Megabases, by genotyping several large families and comparing this data to previously reported information. *In silico* analysis of the region identified 65 transcripts comprising both known genes and putative coding sequences. The transcripts were subdivided into three categories; 26 known genes, 13 putative genes and 26 putative transcripts comprising segments of uncharacterized open reading frame. Fragments encompassing these 65 transcripts are being generated by RT-PCR of cDNA derived from whole blood and screened by two methodologies; conformation sensitive gel electrophoresis and chemical cleavage of mismatched base pairs. The observation that several genes involved in the signaling pathways between the apical ectodermal ridge and the posterior mesenchyme of the developing limb bud are clustered within this critical region is compelling evidence that the mutation responsible for SHFM3 involves this signaling cascade. The identification of further candidates by Positional Transcript Analysis allows more directed mutational screening by sequencing. To date several polymorphisms have been identified and it is our expectation that this approach may be helpful in identifying the causative mutation in SHFM3.

Localization of the Gene for a Novel Autosomal Recessive Neuromuscular Disease Featuring Tremulous and Myoclonic Dystonia with MRI White Matter Alterations. *M. Kambouris^{1, 2}, S.A. Bohlega¹, D. Trabzuni¹, P. Carroll¹, B.F. Meyer¹.* 1) King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia; 2) Yale University School of Medicine, New Haven CT, USA.

A novel autosomal recessive neuromuscular disorder characterized by tremor of juvenile onset, dystonia, myoclonus, spasticity, preserved cognitive cerebellar and peripheral nervous system functions with diffuse white matter alterations in brain MRI is segregating in two consanguineous sibships, parts of one extended family. Central conduction times for visual, motor and sensory systems are all prolonged. Lysosomal, peroxisomal, mitochondrial and other metabolic abnormalities were excluded. Homozygosity mapping localized the offending gene to Hsa 17p33 with LOD=3.89 at Q=0. The expected homozygous genotype in all affected individuals was for markers D17S1298 and D17S1537. Additional mapping is currently performed for narrowing of the linkage interval with microsatellite markers and Single Nucleotide Polymorphisms (SNPs). The linkage interval boundaries at present (9.5 million bp at the physical map, contig #NT_010692.7) are defined by the presence of heterozygosity for an informative distal SNP in affected individuals within the MYO1C gene (an excluded positional candidate) and genetic recombination for the proximal marker D17S974. Positional candidate genes screened for pathogenic sequence alterations include i) GABA (A) receptor-associated protein that clusters Gamma-aminobutyric acid (A) neurotransmitter receptors (ligand-gated chloride channels that facilitate inhibitory neurotransmission) by mediating interaction with the cytoskeleton. ii) Synaptobrevin 2 (VAMP2) vesicle associate membrane protein 2 (part of a protein complex involved in the docking and/or fusion of synaptic vesicles with the presynaptic membrane. iii) CHRN (Cholinergic receptor, nicotinic, binds acetylcholine and opens an ion-conducting channel across the plasma membrane) both E and B1 subunits. Mutation screening is by Mutation Detection Enhancement (MDE) Heteroduplex analyses and sequencing. The unique phenotype and localization to 17p33 are consistent with the identification of a novel neuromuscular disorder.

ARH and HCHOLA3: Two different genes at 1p both implicated in familial hypercholesterolemia. *M. Varret¹, M. Abifadel^{1,2}, L. Villegier¹, A. Robin¹, D. Allard¹, M. Devillers¹, JP. Rabes^{1,3}, C. Junien^{1,3}, C. Boileau^{1,3}.* 1) INSERM U383, Hosp Necker-Enfants Malades, Paris, France; 2) University Saint-Joseph, Beirut, Lebanon; 3) Hosp Ambroise Pare, Paris, France.

Familial hypercholesterolemia (FH), one of the frequent monogenic disease is of autosomal dominant transmission in the vast majority of cases and mainly caused by mutations in the low density lipoprotein receptor gene (LDLR) or in the gene encoding its ligand: apolipoprotein B. However we have shown that more than 30% of autosomal dominant hypercholesterolemia (ADH) cases are not associated with mutations in either these two loci. We reported one large French autosomal dominant pedigree (HC2) in which we excluded linkage to the LDLR and APOB genes and mapped a new locus called HCHOLA3 (previously FH3). The HCHOLA3 gene is now located in a 1.6cM interval between D1S211 and D1S2130 at 1p34. Recently, Garcia et al. described autosomal recessive hypercholesterolemic families with mutations in a new gene localized on chromosome 1: the ARH gene that encodes an adaptor protein probably interacting with the LDL receptor. ARH is located at 1p35 between markers D1S2885 and D1S1152. However, its precise localization is not known because the regional contig is incomplete. The genetic proximity of the ARH gene to the FH3 locus intrigued us. Thus we investigated a possible identity of these two genes by linkage analysis, and systematic sequencing of the ARH gene in affected subjects of the HC2 family. We genotyped 9 microsatellite markers in the region between FH3 and ARH and found no common haplotype segregating with ADH in the HC2 family. No mutation was detected by Southern blot analysis and by sequencing the ARH cDNA of affected subjects in HC2 family. Furthermore, we identified the P202S polymorphism. Although Garcia identified a missense mutation (P202H) at the same codon in one of ARH families, the P202S variation did not segregate with the trait in HC2 and was also found in normolipidemic subjects with a frequency of 0.55 among 100 subjects tested. In conclusion, our results show that despite the fact that ARH and FH3 are in neighbouring genetic regions, they are two different genes implicated in FH.

Genome screen of Alzheimer's disease in a recent genetically isolated population. *C.M. Van Duijn, K. Sleegers, P. Heutink, G. Roks, J. van Swieten, P. Snijders, L. Sandkuijl, B.A. Oostra.* Epidemiology & Biostatistics and Clinical Genetics, Erasmus MC, Rotterdam, Netherlands.

Genetically isolated populations may yield opportunities to identify genes involved in complex genetic disorders such as Alzheimer's disease (AD). Although there is ongoing debate on the use of genetically isolated populations such as the Finnish, recently isolated populations have shown large extents of linkage disequilibrium between markers. To localise new susceptibility genes for AD in a recent genetically isolated population in the Netherlands. The study population comprised a genetically isolated Dutch village of 20.000 inhabitants. The population was founded 300 years ago by 150 subjects and is characterized by rapid growth and minimal immigration. We ascertained 150 AD patients, of whom at least 79% were related within 14 generations. For the genome scan, 44 cases with probable AD (mean age 71.6) were selected who were related to each other within 8 generations. A full genome screen was performed using 420 markers at a marker spacing of 10 cM. The data were analysed using a modification of the method of Terwilliger taking into account patient relationships. Further, we used homozygosity mapping in clusters with (multiple) loops in the pedigree. Preliminary analysis of the genome scan shows positive findings at two markers flanking APOE. In total 16 regions were found with similar p-values or lower. Using a cut off p-value of 0.05, positive hits were also found for chromosomes 3, 4, 6, 7, 10, 11, 12, 14 and 18. At present we are fine typing the identified areas to minimize the regions further. Our preliminary analyses suggest that recent genetically isolated populations are a useful tool for identifying regions of interest that might include new susceptibility genes for AD.

A Targeted Linkage Scan for Nonsyndromic Cleft Lip and Palate. *R.E. Schultz¹, M.E. Cooper², E.K. O'Brien¹, K.A. Graf¹, M.L. Marazita², J.C. Murray¹.* 1) Dept Pediatrics, U of Iowa; 2) School Dental Med, U of Pittsburgh.

Cleft lip and palate (CL/P) is a complex multifactorial disorder present in 1/1000 live births. About 70% of cases are nonsyndromic (NS), with no other associated abnormalities. Fifteen loci were examined for linkage to NS CL/P. Loci were chosen based on previous suggestive findings and include 1p36, 2p13, 4p16, 4q31, 6p23, and 14q24. Polymorphic markers in these regions were genotyped on 36 Filipino families comprising 344 individuals with 126 affecteds. Parametric linkage analysis was performed by FASTLINK, and nonparametric analysis was done by SIMIBD 1.21. FBAT 1.2 carried out the TDT analysis. Five markers yielded suggestive results on the 36 families: the MSX1-CA repeat on 4p16, the microsatellite repeat markers GATA8A05 on 4q31 and D6S1029 on 6p23, and SNPs within the TGFA (2p13) and RFC1 (21q22) genes. MSX1-CA gave a maximum LOD of 1.20, a SIMIBD p-value of 0.2, and a TDT multiallele p-value of 0.76. D6S1029 had a maximum LOD of 0.31, a SIMIBD p-value of 0.06, and a TDT multiallele p-value of 0.76. GATA8A05 had a maximum LOD of 1.12, a SIMIBD p value of 0.244, and a TDT multiallele p value of 0.22. When divided into CL and CLP families, the LOD for GATA8A05 in the CLP group increased to 2.05. RFC1 yielded no positive LOD scores for the 36 families, but had a SIMIBD p-value of 0.088. A TGFA SNP had a maximum LOD of 0.44 but more noteworthy was the SIMIBD p-value of 0.01. The parametric LOD scores for MSX1-CA and GATA8A05, and the SIMIBD p-values for D6S1029, and RFC1 are suggestive while the SIMIBD p-value of 0.01 for TGFA is significant. Genotyping is underway on more markers in these regions and on an additional 70 families with 149 affecteds for validation of these results. Since the *Msx1* mouse knockout has cleft palate and MSX1 mutations have been found in rare cases of syndromic CL/P, this locus is plausible for linkage. Previous studies have also found evidence of linkage of NS CL/P to 4q31 and 6p23, and there are several candidate genes in these regions, including AP2 on 6p23 and FGF2, MADH1, and BMPR1B on 4q31. TGFA has long been considered a candidate gene for NS CL/P. This research was supported by NIH grant DE-08559.

Isolation of novel microsatellite markers on chromosome 6p12-q12 region after *in silico* search of human genome sequence databases. J.-I. Kim¹, H. Rhee¹, Y.-M. Lee², J.A. Park², J.-S. Lee^{1,2}. 1) Brain Korea 21 Project, Yonsei University College of Medicine, Seoul, Korea; 2) Department of Clinical Genetics, Yonsei University College of Medicine, Seoul, Korea.

Microsatellite markers are still useful tools in genome analysis including DNA diagnosis. In addition, it may also provide more valuable information than SNP markers for some genes. In the present study, we have tried to develop new microsatellite markers within chromosome 6p12-q12 region using human genome sequence databases. Human genome sequence databases at UCSC and NCBI were used for *in silico* search of candidate regions of microsatellite markers. Computer program eTRF(Exact Tandem Repeats Finder) was developed to investigate the chromosomal region of 20Mb. After *in silico* search, 61 regions were identified as possible microsatellite markers that contain (CA) or (TG) repeats more than 20 times. Twenty-one among 61 regions which were found to be unique in human genome were selected for experimental validation. PCR primers were designed from flanking sequences of each region and heterozygosity were obtained with 50 individuals among Korean population. All of 21 regions revealed containing microsatellite markers. Heterozygosities were between 0.4 - 0.9 and the maximum difference among the alleles were 12bp on average in Korean population. Many disease genes are mapped to chromosome 6p12-q12 such as congenital nystagmus 2, cone-rod dystrophy 7, Leber congenital amaurosis and dilated cardiomyopathy. Our results would be useful resources to facilitate the identification of these disease genes.

Program Nr: 1601 from 2002 ASHG Annual Meeting

A system approach to high throughput microsatellite analysis. *A. Wheaton, L. Joe, S. Bay, B. Johnson, J. Briggs, C. Brown, O. Ostadan, T. Hatch, Y. Lou.* Applied Biosystems, Foster City, CA.

The completion of the human genome project has introduced a new paradigm in human disease research. With an increasing emphasis on complex diseases, investigators need cost effective, precise tools to rapidly scan the genome for variations. Researchers using traditional methods for high throughput microsatellite analysis face multiple obstacles, such as assay design, sample loading and detection, and data interpretation. Applied Biosystems has taken a system-wide approach to remove these barriers by: developing a pre-configured assay (the ABI PRISM® Linkage Mapping Set v2.5); and introducing a production level capillary electrophoresis instrument, along with highly automated analysis software. The Applied Biosystems 3730 DNA Analyzer, a 48 capillary system, offers the ideal platform for high throughput microsatellite analysis. With the capacity for 48 runs per day, the 3730, in concert with 5-dye chemistry, can generate over 46,000 genotypes per day. The system is completed with GeneMapper™ version 3.0, a fully integrated analysis solution that simplifies the allele calling process. We describe the benefits of this system for high throughput automated microsatellite analysis.

Mapping of a gene for juvenile nephronophthisis to chromosome 1p36. *S. Saunier¹, G. Mollet¹, R. Salomon¹, O. Gribouval¹, F. Silbermann¹, C. Antignac^{1,2}*. 1) Inserm U423, Necker Hospital, Paris, France; 2) Department of Genetics, Paris 5 University, Necker Hospital, Paris, France.

Nephronophthisis (NPH) is an autosomal recessive kidney disorder representing the most frequent inherited cause of chronic renal failure in children. Modifications of the tubules with widening of the basement membrane, interstitial fibrosis and later on, cysts at the cortico-medullar junction are the prominent histological features of the disease. Three loci have been identified, one on chromosome 2q13 (*NPHP1*) for the more common juvenile form, and 2 on chromosomes 9q22 (*NPHP2*) and 3q21 (*NPHP1*) for the infantile and adolescent forms respectively. *NPHP1* encodes a protein (nephrocystin) that contains an SH3 domain and which is implicated in cell-cell and cell-matrix interaction processes through interaction with intracytoplasmic proteins. Since genetic heterogeneity has been shown in juvenile NPH, we undertook a genome-wide linkage analysis in 8 families that were excluded from the 3 known NPH loci and identified a new disease locus, *NPHP4*, on chromosome 1p36. A total of 395 markers were analyzed. We found no evidence of linkage to the 2q13 locus neither to the 3q21 and 9q22 locus previously reported, while two adjacent markers *DIS214* and *DIS2667* on chromosome 1p36 demonstrated segregation with the disease haplotype. The evidence of linkage was highly significant with a maximum two-point lod score for *DIS214* (lod score: 5.3, $q = 0.05$). Haplotype analysis identified a gene interval of 8.7-cM. These results confirm the recent data by Schuermann et al. (*Am J Hum Genet*, 2002, 70:1240-47). Mutational analysis of candidate genes in this region is currently performed in order to identify a new causative gene for NPH. Interestingly, in one of the family linked to this new locus, the 3 affected children present with NPH and Cogan syndrome, a rare neurological disorder, characterized by oculo-motor apraxia and vermis hypoplasia, suggesting a pleiotropic effect of this gene as it has been shown for *NPHP1*. Of note, 4 families were not linked to this locus indicating further genetic heterogeneity.

Program Nr: 1603 from 2002 ASHG Annual Meeting

Three model SNP genotyping assays for human disease: venous thrombosis, breast cancer, and familial Mediterranean fever. *M.D. Dobbs, R.M. Oda, Z. Jiang, S.K. Boyer.* Biological Systems Organization, Beckman Coulter, Inc., Fullerton, CA.

Single nucleotide polymorphisms (SNPs) are the subject of many novel technology development activities because of their high genome density. The ideal SNP genotyping assay will be very accurate, inexpensive, easy to perform, and capable of high throughput. Single-base primer extension utilizes the inherent accuracy of DNA polymerase to determine the presence or absence of specific nucleotides at SNP sites. Single-base extension can also be utilized on several different hardware platforms, and has the advantage of being easily multiplexed. The CEQ™ DNA Size Standard 80 and CEQ™ SNP-Primer Extension Kit were developed for Beckman Coulter's CEQ™ 8000 Genetic Analysis System to provide CEQ users an accurate, inexpensive, simple, and robust solution for multiplex SNP scoring and validation based on single-base extension technology. We present three model multiplex SNP assays for important human diseases: Venous Thrombosis (coagulation factor V gene), Breast Cancer (*brca-1* and *brca-2* genes), and Familial Mediterranean Fever (*MEFV* gene). The assays demonstrate high accuracy and improved throughput through automation and compare favorably with other techniques. The SNP protocols on the CEQ are model methods that can be applied to any other set of SNP polymorphisms.

Implementation of High Throughput SNP genotyping. *J.M. Moore¹, B. Blumenstiel¹, M. DeFelice¹, J. Roy¹, H. Nguyen¹, N. Hattangadi¹, A. Lochner¹, M.J. Daly¹, D. Altshuler^{1,2}, S.B. Gabriel¹.* 1) Center for Genome Research, Whitehead Institute, Cambridge, MA; 2) Departments of Genetics and Medicine, Harvard Medical School; Department of Molecular Biology, Massachusetts General Hospital, Boston, MA.

Our goal is to implement a high quality, scaleable SNP genotyping pipeline. We present data on three aspects of MassArray (Sequenom) platform. First, we have carried out production level genotyping. As part of the TSC Allele Frequency program we scaled SNP genotyping over eight-fold during 2001, averaging 720,000 genotypes per month (80% assay pass rate) over the last six months. Over 15,000 SNPs from the public map were genotyped in multiple population samples. We find an error rate of <0.4% based on reproducibility in duplicate genotypes and inheritance inconsistencies in families. Overall 83% of assays attempted passed all quality checks and were successfully genotyped.

Second, to assess pooled genotyping as a method to rapidly validate candidate SNPs, we implemented a process for genotyping on pooled DNA samples. We have rapidly screened over 10,000 SNPs with this approach across three populations and have directly compared allele frequency estimates from DNA pools to actual frequencies determined from individual genotypes for over 1,000 assays. We find the standard deviation of discrepancy between the measures is 0.05.

Third, we carried out a comparison of the MassArray platform to two additional platforms, Invader (Third Wave) and Taqman (Applied Biosystems). Using a common set of 192 SNPs, we attempted to design assays and genotype a single set of 96 DNA samples. Of assays successfully designed, Sequenom offered the highest rate of assay conversion (86%), with the others producing similarly robust assay design of 75% and 80% by Invader and Taqman, respectively. Error rates were acceptably low in all three methods (<0.5%). In conclusion, we have successfully implemented individual and quantitative genotyping on MassArray and find that this platform, as well as the others evaluated offer robust assay design and accurate genotyping.

Program Nr: 1605 from 2002 ASHG Annual Meeting

Evaluation of new microsatellite analysis software on the MegaBACE platform using multiple populations and marker panels. *E. Shipstone, J. Ellerbrock, D. Meyers, A. Merla, M. Reagin, P. Herzer.* Genomics Applications, Amersham Biosciences, Piscataway, NJ.

A new microsatellite analysis software package, Genetic Profiler v2.0 was evaluated using multiple populations and marker panels. Samples from Amish, Ashkenazi and Mormon populations were used to test the analysis capabilities of the software. Custom marker panels were designed using the software's marker panel editor feature. Markers from various regions of chromosomes 10, 14 and 15 were used to evaluate the capability of the software to identify and characterize di-, tri- and tetranucleotide repeats. Genetic fingerprinting, fragment analysis and expression analysis by peak area calculation were performed. Genetic Profiler v2.0 represents an improvement for analyzing microsatellite data on the MegaBACE platform.

Discovery of single nucleotide polymorphisms in eleven genes involved in muscle function: efficiency of a systematic sequencing approach compared to bioinformatic extraction with five SNP databases. *J.M. Devaney, B.T. Harmon, E.P. Hoffman.* Research Center for Genetic Medicine, Childrens National Medical Center, Washington, DC.

Moving on from the completion of the human genome sequence, studies on DNA variation between individuals will contribute further to the understanding of human genetics. For example, single nucleotide polymorphisms (SNPs) can significantly contribute to the characterization of the genes predisposing an individual to muscle hypertrophy. We report a SNP survey of coding and non-coding regions of eleven genes involved in muscle function, by two successive methods. First, we used high-throughput sequencing of 96 ethnically diverse (36 Caucasian samples, 29 African-American samples, 26 Hispanic samples, and 5 Asian samples) samples to examine around 2.4 million bps. Following sequencing, we examined the traces using a combination of PhredPhrap and PolyPhred software packages that enable rapid analysis for sequence variation amongst the 96 samples. Using this method we discovered 23 SNPs (4 non-synonymous). Second, we compared our newly discovered SNPs to the list of SNPs found for the same eleven genes in five available SNP databases: Celera SNP database, jSNP, dbSNP, HGVbase, and HGMD. Interestingly, we discovered eleven SNPs (three non-synonymous) in the cardiac ankyrin repeat protein gene and none of the SNPs discovered are present in the SNP databases. Therefore, identification of variation in genes should involve high-throughput sequence analysis combined with database searching.

Fine-mapping the bipolar affective disorder locus on chromosome 18q22: A report from the half-way point. *Y-S. Chen¹, N. Akula¹, T. Schulze¹, J.R. DePaulo², N. Cox¹, J. Badner¹, K. Hennessy¹, T. Nguyen¹, F.J. McMahon¹.* 1) University of Chicago, Chicago, IL (USA); 2) Johns Hopkins University, Baltimore, MD (USA).

Several studies have detected evidence of genetic linkage between bipolar affective disorder and chromosome 18q22. Linkage analysis of our clinically-selected subset of families also supports linkage in this region and defines a 5 MB interval containing 10 consecutive microsatellite markers with lodscores >3 . Sequencing in this region is still in progress, encompassing a 60-BAC contig with 3 gaps, 5 known, and up to 25 predicted genes. In order to determine the genetic variation that accounts for the linkage evidence in this region, we are performing systematic linkage disequilibrium mapping with SNPs at 25 kb density. All SNPs are genotyped in 100 cases and 100 ethnically-matched (CEPH) controls by single-base extension methods. We have now completed genotyping and association analysis on 86 SNPs at a median intermarker distance of 58 kb. Linkage disequilibrium analysis between adjacent markers reveals D' values greater than 0.3 across 53% of the region. The majority of SNPs show no evidence of association. Three widely-spaced SNPs show evidence of association at the $p < 0.05$ level. One SNP shows evidence of association at the $p < 0.01$ level, and all of the linkage evidence in this sample is contained in those 17 families segregating the associated allele. Five nearby SNPs spanning a 10 kb interval also show evidence of association at the $p < 0.05$ level, but none of these SNPs partitions the linkage evidence or increases the evidence of association in haplotype-based analysis. These findings do not convincingly account for the linkage evidence on 18q22, but may warrant replication testing in other samples.

Pharmacogenetic analysis in a Gleevec™/Glivec® study of patients with CML identifies an association between fluid retention and a polymorphism in IL1B. *R. Malinowski¹, R. Capdeville², I. Gathmann², S. Kudaravalli¹, M.H. Polymeropoulos¹, M.A. Dressman¹ and IRIS (International Randomized IFN vs STI571) Study Group.* 1) Pharmacogenetics, Novartis Pharmaceuticals Corporation, Gaithersburg, MD; 2) Novartis Pharma AG, Basel, Switzerland.

Gleevec™/Glivec® (imatinib, STI571), an inhibitor of the *bcr-abl* tyrosine kinase, has been shown to be highly effective in the treatment of chronic myelogenous leukemia (CML). Fluid retention was a frequent adverse event involving Gleevec™, occurring in 47-56% of patients in three Phase III clinical trials. Fluid retention described here generally consists of periorbital and facial edema in the skin and subcutaneous region. Pharmacogenetic analysis was completed on a subset of 109 patients from a Phase III study of Gleevec™ versus Interferon- α combined with cytarabine in patients with newly diagnosed previously untreated Philadelphia chromosome positive chronic myelogenous leukemia in chronic phase. We examined 70 SNPs from 26 genes to identify surrogate markers for fluid retention. An association was discovered between the -511 polymorphism in the interleukin-1 beta gene (IL1B) and the adverse event of fluid retention in female patients treated with Gleevec™ ($P = 0.004$). Female patients with the CC genotype for the -511 IL1B polymorphism are 13.0 times more likely to experience fluid retention than those with a non-CC genotype (95%CI 2.07-81.48). The IL1B SNP, representing a C>T transition, is found 511 base pairs upstream from the transcription start site of the IL1B gene. This polymorphism is reportedly in linkage disequilibrium with the -31 polymorphism that functionally relates to a decreased level of expression of the IL1B gene. The association of the autosomal -511 IL1B polymorphism with edema in females is surprising but may be related to the increased incidence of edema observed in women compared to men treated with Gleevec™.

Genetic variants associated with increased susceptibility for Down syndrome-associated Atrioventricular Septal

Defects. *K.F. Kerstann¹, S.B. Freeman¹, E. Feingold², L.J. Bean¹, M. Meltzer³, A. Heffner³, G. Capone^{3,4}, S.L.*

Sherman¹. 1) Dept. of Human Genetics, Emory University School of Medicine, Atlanta, GA; 2) Dept. of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 3) Div. of Neurology and Developmental Medicine, Kennedy Krieger Institute, Baltimore, MD; 4) Dept. of Pediatrics Johns Hopkins Medical Institutions, Baltimore, MD.

Atrioventricular septal defects (AVSD) occur in about 20% of live births with Down syndrome (DS), while in only 3-4/10,000 live births with euploid chromosome complements. This increased risk among individuals with DS must be associated with increased dosage of chromosome 21 specific genes. However, the observation that only 20%, not 100%, of DS individuals have AVSD suggests that genetic variation and/or environmental factors influence the susceptibility of this birth defect. The increased dosage of a gene(s) on chromosome 21 may lead to altered regulation or function of the chromosome 21 specific gene(s) and/or may cause downstream effects on other non-chromosome 21 genes. The overall goal of this project is to identify genes and environmental factors that lead to the susceptibility of AVSD among individuals with DS. We have begun by testing the hypothesis that genes on chromosome 21 play a role in heart development and that when in triplicate, specific allelic forms lead to susceptibility for AVSD. We have focused on 21q22.2-22.3, between markers D21S55 and COL6A2, a proposed critical region for congenital heart defects (CHD) and specifically on genes within this region found to be expressed in fetal heart. Of particular interest is SH3BGR, a gene within the critical region and shown to be expressed during heart development. The genetic variation within SH3BGR is primarily contained within a set of 20 SNPs that fall into 5 previously reported haplotype blocks. This variation is being compared between 54 individuals with DS and complete AVSD(cases) and 92 individuals with DS and no CHD(controls). Newly developed statistical methods for comparison of trisomic genotypes, incorporating a TDT approach will be used to assess differences among cases and controls.

The polymorphisms in the eosinophil cationic protein gene and its influence on the serum ECP level. E.

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Asthma is characterized by reversible airway obstruction and airway inflammation. Serum levels of eosinophil cationic protein (ECP) might reflect eosinophilic airway inflammation and asthma activity. However, the serum ECP levels are not elevated in some asthmatic patients, even when they are symptomatic. In this study, we screened for polymorphisms in ECP and analyzed the associations of these polymorphisms with asthma and serum ECP levels in 137 Japanese families identified through asthmatic children. We identified three polymorphisms (-393C/T, -38C/A and 124Arg/Thr) in human ECP. We did not find associations between these polymorphisms and asthma by transmission disequilibrium test. However, we found that serum ECP levels in subjects with the -393T allele were significantly lower than those in subjects with the -393C allele. A reporter construct with the -393T allele showed significantly lower promoter activity than one with the -393C allele. These data indicate the -393T allele has low transcriptional activity compared with that of the -393C allele and that measurement of ECP levels for the assessment of asthma activity may be improved when done in combination with genotyping of the -393C/T polymorphism.

Haplotype-based association of DRD2 gene to alcoholism with antisocial personality disorder. *K. Xu, J. Tabuman, C.H.D. Bau, M. Virkkunen, M. Linnoila, E. Ferro, W. Astor, D. Goldman.* Lab Neurogenetics, NIAAA/NIH, Rockville, MD.

Because of the role of the D2 receptor in reinforcement, the DRD2 gene has been extensively studied in addictive behaviors, but with conflicting results. One critical problem has been a focus on a limited number [or even one, usually the Taq1A locus] of nonfunctional markers at the gene. To better understand the relationship of DRD2 variants and addictions, we have genotyped [using 5 nuclease assays] DRD2 using a panel of ten loci, including the functional loci Ser311Cys and -141delC, and this panel spans the 80 kb of chromosome 11q23 that contains the entire DRD2 gene region, including a 50 kb intron separating the non-coding first exon from the remainder of the exons. We have completed haplotype-based analyses using four loci including the two functional loci plus Taq1A [10 kb downstream] and Taq1B [located in IVS 1]. Linkage was performed in a Finnish population composed of 63 alcohol dependence (AD) with antisocial personality disorder (ASPD) and 141 unaffected individuals. Frequency of the Taq1B A allele was higher in individuals with AD plus ASPD than in the unaffected group (Chi-square = 7.314, 2 df p = 0.026, for genotype ; Chi-square = 5.298, 1 df p = 0.021 for allele frequency). There was no association of any of the other three SNPs with AD plus ASPD. The overall 11 haplotypes comparison between AD plus ASPD and control group was significant different. (Chi-square = 23.47 10 df p = 0.009). Moreover, the haplotype CACG was more abundant in AD with ASPD group (0.188) than in the unaffected group (0.067) (Chi-square = 7.689 1df p = 0.006). Taken together, we conclude that the DRD2 gene may be a susceptibility gene for AD with ASPD in a Finnish population, warranting efforts to close in on the locus that may be influencing vulnerability through altered DRD2 function.

Analyses on the association of Fcγ receptor family and *TNFR2 (TNFRSF1B)* polymorphisms with susceptibility to rheumatoid arthritis in Japanese. C. Kyogoku¹, N. Tsuchiya¹, K. Matsuta², T. Shibue¹, K. Tokunaga¹. 1) Dept of Human Genetics, The University of Tokyo, Tokyo, Japan; 2) Matsuta Clinic, Tokyo, Japan.

We recently reported a new polymorphism of *FcγRIIB* gene (*FCGR2B*), which alters Ile at position 232 to Thr (*I232T*) within the transmembrane domain, and its association with SLE in Japanese. Functional importance of *FcγRIIB*, as well as other *FcγRs*, in RA has previously been shown; therefore, we examined whether *FCGR* polymorphisms were associated with susceptibility to RA in Japanese. *TNFR2* is another candidate gene located on one of the chromosomal regions implicated from genome-wide linkage studies, and recent studies demonstrated that *TNFR2-196R/R* genotype was significantly increased in familial RA in Caucasians. Our previous study showed a tendency of increase of *196R/R* genotype in Japanese sporadic RA ($P=0.10$). Therefore, we genotyped additional patients and controls to further test the association of *196R/R* in Japanese.

Genotyping of *FCGR2B-I232T*, *FCGR2A-H131R*, *FCGR3A-F176V*, *FCGR3B-NA1/2* and *TNFR2-M196R* polymorphisms were performed in 382 Japanese patients and 303 healthy individuals for *FCGRs*, and in 588 patients and 308 controls for *TNFR2*.

Significant difference in the distribution of genotype, allele carrier and allele frequencies was not observed between RA and controls in *FCGR* genes. However, when the subjects were stratified according to the carriage of *HLA-DRB1* shared epitope (SE), significant increase of *FCGR3A-176F/F* genotype was observed in SE positive RA (55.9%) compared with SE positive controls (41.5%) ($P=0.009$).

As for *TNFR2*, a tendency of increase of *196R/R* genotype in RA (2.9%) compared with controls (1.0%) was observed ($P=0.07$). However, estimation of genotype relative risk, which adjusts control data for Hardy-Weinberg equilibrium, indicated significant association of *196R/R* genotype with RA ($c^2=6.2$, $P=0.01$, OR:2.3).

These results indicated that *FCGR3A-176F/F* genotype confers risk for RA through genetic interaction with *HLA-DRB1* SE, and *TNFR2-196R/R* genotype may be a weak, but true, risk factor in the sporadic RA, in Japanese.

Examination of Candidate Genes on Chromosome 7 in Autism. *H.B. Hutcheson¹, L.M. Olson¹, Y. Bradford¹, S.E. Folstein², S.L. Santangelo³, J.S. Sutcliffe¹, J.L. Haines¹.* 1) Dept. Molecular Physiology and Biophysics, Program Human Genetics, Vanderbilt University Medical Center, Nashville, TN; 2) Dept. of Psychiatry, New England Medical Center/Tufts University School of Medicine, Boston, MA; 3) Psychiatric & Neurodevelopmental Genetics Unit, Massachusetts General Hospital, Boston, MA.

We previously reported a method for examining haplotype data in the CLSA chromosome 7-linked families to narrow the most likely chromosome 7 autism candidate region (*AUTS1*) to a 3 cM interval located between D7S496-D7S2418. In this study, we chose to examine 4 candidate genes (*NRCAM*, *LRRN3*, *KIAA0716*, *LAMB1*) based on their proximity to the microsatellite marker corresponding to the highest LOD score in this subset of thirty families (D7S1817), their expression patterns, and their biological relevance to autism. We analyzed 36 intronic and exonic single nucleotide polymorphisms (SNPs) and one microsatellite marker spaced at ~10kb intervals within and around these 4 candidate genes. This was done to provide as complete coverage as possible since linkage disequilibrium (LD) can vary dramatically across even very short distances within a gene. Association analysis included both single locus (PDT) and multilocus (TRANSMIT) approaches. We also examined the extent of LD within these genes and across this region. As expected, we observed blocks of significant LD within each of these genes but did not observe significant LD across genes since these genes span a distance of 3.5Mb. None of the polymorphisms in *NRCAM*, *LRRN3*, or *KIAA0716* singly or in combination gave *P* values <0.05 suggesting that none of these genes is associated with autism susceptibility in this subset of chromosome 7-linked families. However, analysis of *LAMB1*, which encodes a protein that has an integral role in embryonic axonal guidance, revealed suggestive evidence for allelic association, including one individual SNP (*P*= 0.02) and three separate two-SNP haplotypes across the gene's transcriptional unit (0.007, 0.012, and 0.012). Currently, we are screening the 34 exons of *LAMB1* for susceptibility variants in autistic individuals.

An autoimmune diabetes locus (*Idd21*) on mouse chromosome 18. *R.J. Hall¹, J.E. Hollis-Moffat¹, M.E. Merriman¹, R.J. Bridges¹, R.A. Green¹, G. Williams¹, D. Baker², T.R. Merriman¹.* 1) Biochemistry Department, University of Otago, Dunedin, New Zealand; 2) Department of Neuroinflammation, Institute of Neurology, University College London, London, UK.

Autoimmune diabetes occurs spontaneously in the non-obese diabetic (NOD) mouse and is used to model human type 1 diabetes (T1D). The condition is precipitated by the action of multiple insulin-dependent diabetes (*Idd*) genes which cause a loss of immune self tolerance and subsequent destruction of insulin-secreting pancreatic b-islet cells in the NOD mouse. Genes of the major histocompatibility complex (MHC) are the major genetic determinant of disease with at least twenty other non-MHC loci contributing to a lesser extent. A meta-analysis of genome-wide linkage scans suggests the presence of an autoimmune disease locus on distal mouse chromosome 18 - this region is orthologous to human chromosome 18q12-q21 (which contains the T1D locus *IDDM6*) and rat distal chromosome 18 (which contains the T1D locus *Iddm3*). In this study we present evidence for linkage of mouse chromosome 18 to diabetes in a (ABH x NOD)F1 x NOD backcross. A consomic strain of NOD with chromosome 18 derived from the diabetes resistant strain ABH was generated (NOD.ABH-Chr18). These mice show a delayed onset and an overall reduced frequency of diabetes when compared to the parental NOD mice ($P < 0.0001$). However, no difference in the amount of inflammation within the endocrine pancreas was observed at 8 - 9 weeks of age. Our data confirms the presence of a diabetes susceptibility locus on mouse chromosome 18, which we have named *Idd21*.

Investigation of Human Chromosome 3q for a Major Susceptibility Gene for Diabetic Nephropathy using Linkage Disequilibrium Mapping. *D.P.K. Ng, J.J. Rogus, A. Antonellis, L.H. Canani, Y. Makita, D. Moczulski, M. Nam, W.H. Walker, A. Smiles, J.H. Warram, A.S. Krolewski.* Section on Genetics and Epidemiology, Joslin Diabetes Center, Boston, MA.

Strong evidence from linkage studies supports the presence of a major susceptibility gene for diabetic nephropathy (DN) on human chromosome 3q. Recently, we undertook a systematic search of a 1.2 Mb chromosomal region which contains a number of positional candidate genes for DN. These include genes for angiotensin II type 1 receptor (*AGTR1*), carboxypeptidase A3 (*CPA3*) and carboxypeptidase B1 (*CPB1*). Based on our physical map (also supported by Human Genome Project and Celera Genomics draft assemblies), these genes are encompassed within a ~750 kb region. To scrutinize this promising region, 48 single nucleotide polymorphisms, 4 insertion/deletions and 5 microsatellites were developed. Allele frequencies were compared between 94 type 1 diabetic patients with advanced DN (Cases) and 94 patients who have remained normoalbuminuric despite >15 years of diabetes (Controls). Distortion in allele distribution was observed for six markers (markers 34, 35, 37, 38, 41, 54) ($P < 0.05$). The region was next partitioned into haplotype blocks (Daly et al. 2001) as defined by the occurrence of historical recombinations. Adapting the algorithm of Hudson and Kaplan (1985), 20 blocks were defined in this region. Frequencies of common (i.e. >5%) haplotype tags (Johnson et al. 2001) in each block were compared and significance was assessed empirically. Subsequent 2-marker haplotype analyses revealed that the '2-1' haplotype formed from markers 37 and 38 (both in *CPB1* locus but residing in separate haplotype blocks), was more prevalent among Cases (41%) than Controls (25%) (nominal significance after adjustment for multiple comparisons). Our ongoing work involves confirming positive markers using larger Case-Control studies and TDT analyses. Strategies are currently being developed to test 'super-haplotypes' derived from combinations of individual block haplotypes. This approach allows for the possibility that the DN susceptibility allele(s) may be associated with only a subset of common haplotypes within a given block.

Genetic Linkage of Francois-Neetens fleck (mouchetée) Corneal Dystrophy to Chromosome 2q35. X. Jiao¹, F.L. Munier², D.F. Schorderet², B.I. Rubin¹, J. Smith¹, J.F. Hejtmancik¹. 1) National Eye Inst, NIH, Bethesda, MD; 2) Oculgenetic Unit, Jules Gonin Eye Hospital and Division of Medical Genetics, Lausanne, Switzerland.

Francois-Neetens fleck (mouchetée) corneal dystrophy is an autosomal dominant corneal dystrophy characterized by scattered small clear flecks occurring at all levels of the corneal stroma. The number of flecks usually varies from tens to hundreds, but can be rare and the flecks can even occur unilaterally. Clinically, although CFD may occasionally cause mild photophobia, patients are typically asymptomatic and have normal vision, the disease most often being found on routine examination. We report linkage of the CFD locus to D2S2289 ($Z_{\max} = 5.92$, $q = 0$), D2S325 ($Z_{\max} = 4.67$, $q = 0$), D2S317 ($Z_{\max} = 3.68$, $q = 0$), D2S143 ($Z_{\max} = 4.37$, $q = .03$), and D2S2382 ($Z_{\max} = 4.71$, $q = 0$) on chromosome 2q35. Multipoint analysis confirmed linkage to the region between D2S117 and D2S396 with a maximum multipoint lod score of 6.38 located at D2S157. Candidate genes in this region are currently being screened for mutations.

Transmission/disequilibrium tests of microsatellite polymorphic markers nearby skeletal muscle ion channel genes in Thai families with thyrotoxic periodic paralysis. *W. Jongjaroenprasert¹, A. Al-chalabi¹, S. Nakasatian³, B. Ongphiphadthanakul⁴, R. Rajatanavin⁴, T. Himathongkum³, R.G. Dluhy², R.H. Brown¹.* 1) Cecil B Day Laboratory for Neuromuscular Research, Massachusetts General Hospital East, Charlestown, MA02129, USA; 2) Division of Endocrinology and Hypertension, Brigham and Women's Hospital, Boston, USA; 3) Theptarin Hospital, Bangkok, Thailand; 4) Endocrine Unit, Ramathibodi Hospital, Mahidol University, Thailand.

Thyrotoxic periodic paralysis (TPP) is characterized by periodic episodes of hypokalemia and weakness in thyrotoxic patients. It has been predominantly described in male Oriental population. Clinical features are similar to familial hypokalemic periodic paralysis (FPP). Defects in genes encoding three types of skeletal muscle ion channels have been implicated in FPP. We have therefore performed a family-based study to test the hypothesis that alleles defined by microsatellite polymorphic markers within 1cM of these three ion channel genes are over-represented in TPP which would potentially implicate the associated ion channels in the pathogenesis of TPP. Genotyping of Microsatellite markers within 1 cM of the genes coding for ATP-sensitive potassium channel (KCNJ11), Kir-related peptide (ABCC9), voltage-gated potassium channel (KCNC4), Mink-related peptide 2 (KCNE3), alpha-1s subunit of the L-type voltage-dependent calcium channel (CACNA1S) and the alpha subunit of the voltage dependent, type IV, sodium channel (SCN4A) were obtained using an automated DNA sequencer (Licor) with and RFLP Scan software. The significance of association of specific alleles with the TPP trait was evaluated by TDT and sib-TDT. Pedigrees of 30 Thai TPP patients were studied. All patients were males with thyrotoxicosis secondary to Graves disease. High serum thyroid hormone and low potassium levels were confirmed during weakness. Genotypes were obtained on 133 individuals (30 cases and 103 family members). We did not detect evidence of association or over-representation of any of the above microsatellite polymorphic markers with the TPP trait in these 30 pedigrees. These data suggest that polymorphisms or variants within these six genes are not related to the pathogenesis of TPP.

An E23K SNP in the islet ATP-sensitive K⁺ channel gene (Kir6.2) contributes to the risk of type 2 diabetes in Caucasians. *L. Love-Gregory¹, J. Wasson¹, J. Lin², G. Skolnick¹, B. Suarez², M. Permutt¹.* 1) Div Metabolism, Washington Univ Sch of Medicine, St Louis, MO; 2) Psychiatry-Instruction & Research, Washington Univ Sch of Medicine, St. Louis, MO.

It is still unknown as to whether common or rare variants are the culprits in the development of type 2 diabetes (T2D) and if these susceptibility loci will have a single allele altering risk or if combinations of these variants will affect risk. One such common polymorphism occurs in Kir6.2 (KCNJ11 gene on 11p15.1), a subunit of the inwardly rectifying ATP sensitive K⁺ channel (involved in the regulation of insulin secretion in pancreatic b-cells). In Hani EH et.al., *Diabetologia* (1998), we reported an association between the Kir 6.2 E23K polymorphism (KK) genotype and T2D in a case-control meta-analysis. Recently, Schwanstecher C, et al., *Diabetes* (2002) demonstrated a dose-dependent reduction in ATP sensitivity of the Kir6.2 E23K variant. Hence, we decided to re-evaluate the common E23K polymorphism by genotyping an additional population (Ashkenazi Jewish-unpublished) and including the UKPDS data. Here we assessed the relative risk associated with the (K) allele and the homozygous E23K (KK) status.

The results of this analysis showed that the homozygous E23K (KK) genotype was more frequent in the T2Ds than the control group [0.18 (n=1153) vs 0.11 (n=1076), respectively, corrected p=1.0 x 10⁻⁴]. When the relative risks from the 6 studies were weighted by their sampling variance, meta analysis indicated that the K allele increases risk by an average of 13% (95% CI: 1.07 to 1.18) which translates into an average attributable relative risk of 11.4% for the populations in these studies. This suggests that if this sample population were monomorphic for the non-risk allele (E), the prevalence of T2D would be 11.4% lower in this group. The KK homozygote is at greatest risk (RR=1.28, 95% CI: 1.19 to 1.37). This confirms that the E23K (KK) genotype increases susceptibility to T2D and is comparable to the risk associated with PPARγ Pro12Ala (a variant that has consistently been shown to be associated with an increase in risk of T2D).

Association Studies of Apolipoprotein AV and Familial Combined Hyperlipidemia. *R.A. Mar¹, H. Allayee¹, G.M. Dallinga-Thie², R.M. Cantor¹, T.W.A. de Bruin³, A.J. Lusi¹.* 1) Dept. of Human Genetics, UCLA, Los Angeles, CA 90095; 2) Dept. of Medicine, University Hospital, Utrecht, The Netherlands; 3) Dept. of Internal Medicine, University of Maastricht, The Netherlands.

Familial combined hyperlipidemia (FCH) is a complex, heterogeneous disorder characterized by elevated levels of plasma cholesterol and/or triglycerides. Multiple studies have reported linkage to the apolipoprotein (APO) AI-CIII-AIV region on chromosome 11q. The minor alleles of three restriction site polymorphisms, XmnI and MspI located in APOAI and SstI in APOCIII, were previously shown to be more frequent in 18 Dutch FCH probands and hyperlipidemic relatives compared to normolipidemic controls. These alleles were also associated with elevated plasma cholesterol, triglycerides, apoB and apoCIII levels. APOAV, located 30kb downstream of APOAIV, has recently been shown to influence plasma triglycerides in mice and humans. To investigate the role of APOAV in FCH, a promoter and two intronic SNPs were genotyped in 80 Dutch FCH probands and 100 unrelated controls as well as in a US population of 400 combined hyperlipidemics and 230 normolipidemic controls. The minor alleles of the two intronic SNPs, but not the promoter polymorphism, were more frequent in the US combined hyperlipidemics compared to controls ($P < 0.025$ and 0.05). These SNPs were also associated with elevated plasma triglycerides in both combined hyperlipidemics and controls ($P < 0.005$ and 0.01). Although no significant difference in the frequency of the SNPs was observed between the Dutch probands and controls, the XmnI, MspI, and SstI genotypes were used to build haplotypes with the APOAV SNPs. Pairwise measure of linkage disequilibrium between the SstI polymorphism and each of the three SNPs in APOAV yielded $|D'|$ values of 1.00 for all three SNPs in the proband group, but only $|D'|$ values of 0.552, 0.554, and 0.417 in the spouses. The strong LD observed between polymorphisms in APOCIII and APOAV in FCH probands, but not the spouses, suggests that both genes play a causal role in FCH. Taken together, these data further indicate the complex genetic contribution of this gene cluster to FCH as well as lipid levels in the general population.

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Tests for Association for Quantitative and Censored Traits. *O. Mokliatchouk.* Mathematics & Computer Sci, Drexel Univ, Philadelphia, PA.

The intended use of the transmission/disequilibrium test was detecting linkage when population association exists with a particular marker. Recently it also has been used to test for association when linkage between a disease locus and a marker exists. The motivation for such test is the necessity to narrow large candidate regions identified by classical linkage analysis. The modification of the transmission/disequilibrium tests is necessary when the method is used for testing association in the case of data on families with more than one offspring. Such modifications are proposed for the cases of quantitative and censored traits. Results of simulations that explore the performance of the proposed tests are described. The procedures are illustrated through an analysis of data on APOE genotype, a mutation of alpha-2 macroglobulin gene (A2M) and Alzheimer's disease.

Identification and analysis of polymorphisms within STAT1: association with asthma and associated phenotypes.

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Asthma is a chronic inflammatory disorder of the bronchial airways characterized by allergic responses and bronchial hyperresponsiveness. Asthmatic inflammation has been shown to be both directed and propagated by the secretion of a series of specific cytokines. Because cytokine effects often depend on signal transducers and activators of transcription (STATs), STAT1 is a functional candidate gene for influencing inflammatory disease. In addition, STAT1 is located on chromosome 2q33, where evidence of linkage has been observed in genome screens for asthma and associated phenotypes. To evaluate its role in asthma and associated phenotypes, we have sequenced all 25 STAT1 exons in 96 individuals for SNP identification. We performed association studies with over 10 SNPs in Dutch, Caucasian, Hispanic, and African-American asthma populations. We observed significant associations ($p < 0.05$) of several polymorphisms throughout STAT1 with allergy and asthma related phenotypes (e.g., bronchial hyperresponsiveness, FEV1, and log IgE). These data suggest that variations within STAT1 may contribute to the pathophysiology of allergy or asthma.

Analysis of a dense SNP based map in a psoriatic cohort defines genetic variation at PSORS1, the major psoriasis susceptibility locus. *C.D. Veal¹, F. Capon^{1,2}, M.H. Allen³, E.K. Heath¹, J. Evans¹, A. Jones³, S. Patel¹, D. Burden⁴, D. Tillman⁴, J.N.W.N. Barker³, R.C. Trembath¹.* 1) Division of Medical Genetics, University of Leicester, Leicester, England; 2) Division of Human Genetics, "Tor Vergata" University of Rome, Italy; 3) St John's Institute of Dermatology, Kings College, London, England; 4) Department of Dermatology, Western Infirmary, Glasgow, Scotland.

Psoriasis is a common cutaneous disorder characterised by keratinocyte hyperproliferation, immune cell infiltration and angiogenesis. The major susceptibility locus (PSORS1), located within the MHC on 6p21.3, has been refined by linkage disequilibrium (LD) mapping to an approximate 200kb region including genes with reported association to psoriasis (HLA-C, HCR and CDSN). However, data interpretations have been compounded by a lack of detail for the background pattern of LD across the region. In order to identify single nucleotide polymorphisms (SNPs) of relevance to psoriatic disease chromosomes, and to further characterise the LD within this region, we sequenced 64kb in segments across this interval in 8 psoriatic individuals who were heterozygous for previously identified microsatellite high risk haplotypes. A cohort of UK caucasian affected offspring trios (n=171) were genotyped for SNPs displaying a frequency greater than 20% (n=59). Significant deviation ($p < 0.001$) from expected transmission of alleles was observed for SNPs across the entire region, however strongest association was seen for SNPs located 7kb centromeric of HLA-C ($p < 0.00000001$). Examination of haplotypes found these SNPs to be exclusive to over transmitted chromosomes, all other variants in the region were also present on under or neutrally transmitted chromosomes, including those within HCR and CDSN. These data demonstrate the power of SNP haplotype-based association analyses and provide high-resolution dissection of the genetic variation across the PSORS1 interval.

Comparative Genetic Study of Tuberculosis, Asthma and Inflammatory Bowel Disease Susceptibilities. A.H. Poon¹, A. Jiménez-Corona², M. Palacios-Martínez², J. Sifuentes-Osornio³, A. Ponce-de-León³, M. Bobadilla³, M. Kato³, C. Laprise⁶, A. Bitton⁷, P.M. Small⁴, M.L. García García², T. Hudson⁵, E. Schurr¹. 1) Centre for the Study of Host Resistance, McGill University, Quebec, Canada; 2) Instituto Nacional de Salud Pública, Cuernavaca, Mexico; 3) Instituto Nacional de Ciencias Médicas y de Nutrición Salvador Zubirán, Mexico City, Mexico; 4) Medicine, Stanford University, CA, USA; 5) Montreal Genome Centre, Quebec, Canada; 6) Complexe Hospitalier de la Sagamie and University of Quebec at Chicoutimi, Quebec, Canada; 7) Division of Gastroenterology, McGill University Health Centre, Quebec, Canada.

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is the leading cause of death due to a single pathogen. It has been suggested that genetic polymorphisms predisposing to TB are protective for atopy/asthma or inflammatory bowel disease (IBD). We analysed candidate TB susceptibility loci for their association with TB in a Mexican population and tested for inverse genetic associations with asthma and IBD in two large Canadian family panels of both diseases.

The TB case-control study included 643 subjects, the asthma study recruited 182 nuclear families and the IBD study recruited 104 families. We genotyped three *NRAMP1* polymorphisms (c.274C>T, D543N, 1799+del14)(acc.# L32185), the *TNFA*-308A>G polymorphism (acc.# X02910) and two *VDR* polymorphisms (g.12022A>G, g.46160A>G)(acc.# AC004466) by TaqMan assays. Results were analyzed by the chi square test, the combined transmission disequilibrium test (TDT)/sib TDT and the family-based association test (FBAT) program according to study design.

For TB, we observed associations for genotype *VDR*g.46160G/G (p=0.027), allele *VDR*g.12022A (p=0.015) and allele *TNFA*-308A (p=0.047). For asthma, allele *VDR*g.46160A was associated with its subphenotypes (0.072 < p < 0.125). For IBD, allele *TNFA*-308G was associated with disease susceptibility (p=0.036).

Our results provide experimental evidence of the hypothesis that genetic variants predisposing to TB are protective for atopy or IBD and vice versa.

Association of variation between *GABRB3* exon 3 and 155CA-2 with Autistic Disorder. *L.A. Weiss*¹, *D. Gonen*², *S.J. Kim*², *Z. Yang*², *N.J. Cox*¹, *E.H. Cook*². 1) Department of Human Genetics, University of Chicago, Chicago, IL; 2) Department of Psychiatry, University of Chicago, Chicago, IL.

Autistic Disorder (AD) has been shown to be in linkage disequilibrium with *GABRB3* 155CA-2, a dinucleotide marker in intron 3 of *GABRB3*, in two samples (nominal $p \leq 0.001$ in each) but not several other samples (Buxbaum, et al. *Mol Psychiatry* 2002; 7: 311-6; Cook, et al. *Am J Hum Genet* 1998; 62: 1077-83.). Sequence in this intron was independently obtained and several SNPs 3' of *GABRB3* 155CA-2 did not show significant LD (nominal $p > 0.01$) with AD in this sample (Kim, et al. *Am J Hum Genet* 2000; 67 (suppl.): 1773.). The laboratory obtained sequence that filled in the previous ~ 10 kb gap between *GABRB3* exon 3 and *GABRB3* 155CA-2. Direct sequencing of PCR products was performed to screen for variation in 10 AD probands selected for having at least one copy of the overtransmitted *GABRB3* 155CA-2 103 allele. Fifteen SNPs and 4 length variants were discovered by this method. Of the fifteen SNPs and two simple insertion/deletion polymorphisms, only eight unique patterns were observed, and the rest of the variants were in perfect LD with one of these eight markers. Four of these SNPs (representing four of the eight unique patterns) were genotyped by SBE-FP in 92 AD trios, and the two simple length variants were genotyped by amplification with fluorescently labeled primers and capillary electrophoresis. Two variants, which are in perfect LD in this sample, were associated with AD as determined by the Transmission/Disequilibrium Test (local SNP ID: GABRB3-SNP09 and D15S1539 nominal $p=0.0082$). Haplotype TDT analysis of adjacent markers showed p -values for markers GABRB3-SNP09 and GABRB3-SNP11 of 0.001 and for markers GABRB3-SNP05 and D15S1540 of 0.012.

Linkage and segregation analysis of phenotypic subtypes of dyslexia. *N. Chapman*¹, *R. Igo*², *J. Thompson*³, *W. Raskind*¹, *V. Berninger*³, *E. Wijsman*^{1,2}. 1) Dept of Medicine; 2) Dept of Biostatistics; 3) Dept of Educational Psychology, Univ. Washington, Seattle WA.

Reading disability (RD), or dyslexia, has a genetic basis. We are studying quantitative phenotypic components of RD in families ascertained through a proband with RD. Analysis of phenotypic subtypes, along with adjustment for covariates, may have increased power to detect linkage, over the use of qualitative phenotypes. Also, use of the Bayesian Monte Carlo Markov chain methods increases the efficiency of data use. These methods allow multilocus trait models, in the presence of very large numbers of markers in joint linkage and segregation analysis. Pseudoword reading efficiency (PR) and Word-ID (WID) are of particular interest, because of previous reports of evidence of linkage of analogous measures to several genomic regions.

We carried out Bayesian segregation analysis, and Bayesian joint linkage and segregation analysis, for PR and WID. We analyzed chromosomes(ch) 6, 15, and 18, all of which have been reported as showing evidence for linkage to similar RD-related phenotypes. For PR adjusted for age, sex and verbal IQ, the posterior mean number of quantitative trait loci (QTLs) was ~4.1, of which ~1.7 of the QTLs contribute at least 5% to the total variance. When PR is also adjusted for the effects of Word Attack (WA), the number of QTLs contributing to PR drops by ~1. The posterior mean number of QTLs for WID is ~3.6. Linkage analysis of these two measures, using all markers per ch from a 10 cM scan, provided evidence against linkage of PR to ch 6 and 15, and against linkage for WID to ch 6 and 18, with intensity ratios (IR) - posterior to prior rates of accepted models with particular QTL map positions - below 1. For PR, an IR of ~2.7 was found for ch 18 at 70 cM, and for WID an IR of ~3.7 was found for ch 15 at ~50 cM. These results provide weak evidence in support of RD loci on ch 15 and 18, but no evidence of RD loci on ch 6. Additional analyses, using covariates and/or joint analysis of multiple chromosomes will be needed to fully evaluate evidence for linkage of these phenotypes.

Localisation and fine mapping of a recombination hotspot on chromosome 19. *J. Jones*¹, *A. Cox*², *A.G. Wilson*¹. 1) Division of Genomic Medicine, University of Sheffield, Sheffield, South Yorkshire, UK; 2) Institute of Cancer Studies, University of Sheffield, Sheffield, South Yorkshire, UK.

The rate of recombination across the human genome, calculated by comparisons between genetic and physical map distances, has been shown to be highly variable. Recent reports indicate the presence of recombination hotspots, where the rate of recombination is significantly higher than that seen in other chromosomal regions. One indication of the presence of such a hotspot is the consequent breakdown of linkage disequilibrium in the region.

Recent analysis of chromosome 19p13 revealed patterns of linkage disequilibrium indicative of the presence of a recombination hotspot in a 1Mb region, defined by markers D19S406 and D19S427 (McCarthy et al, 2001). Further data shows a disease-locus defining recombination event within this region (Faivre et al, 2002).

Recombination analysis of 19p13 was performed by genotyping both DNA from families and single sperm. Familial analysis is dependent on the availability of samples from both parents and at least 2 children. It is also dependent on the parents being heterozygous for the markers tested in order for inherited haplotypes to be determined. Single sperm typing is a powerful tool that eliminates the need for the collection of extensive family samples, although heterozygosity at the marker of interest is still essential.

Initial genotyping data of 14 informative families has identified 2 recombinations in 28 meioses. This is double the expected recombination rate for a region of this size. Further analysis of 100 families is currently underway in order to increase the power of the study and prove the presence of a hotspot by identification of an elevated recombination rate. The refinement of the region of recombination, including the use of SNP mapping, will also be described.

A fourth locus of postaxial polydactyly type A/B on chromosome 7p11.2-q32. *R.J.H Galjaard¹, A.P. Smits², J.H.A.M. Tuerlings², A. Bais¹, A. Bertoli¹, G. Breedveld¹, E. de Graaff¹, B.A. Oostra¹, P. Heutink¹.* 1) Clinical Genetics, Erasmus Medical Center, Rotterdam, Netherlands; 2) Human Genetics, University Hospital Nijmegen, Netherlands.

Postaxial polydactyly (PAP) is the occurrence of one or more extra ulnar or fibular digits or parts of it. In PAP-A the extra digit is fully developed and articulates with the fifth or an additional metacarpal/metatarsal, while it is rudimentary in PAP-B. Isolated PAP usually segregates as an autosomal dominant trait, with variable expression. Three loci are known for PAP in humans. PAP-A1 (including PAP-A/B in one patient) on 7p13 caused by mutations in the *GLI3* gene. PAPA2 on 13q21-q32 in a Turkish kindred with PAP-A only, and a third one (PAP3) in a Chinese family with PAP-A/B on 19p13.1-13.2. We identified a fourth locus in a large Dutch six generation family with 31 individuals including 11 affecteds. Their phenotype varied from either PAP-A, or PAP-B to PAP-A /B with or without partial cutaneous syndactyly. We performed a whole genome search. Linkage was found between PAP and markers of chromosome 7. The highest LOD score was obtained at marker D7S1799 ($Z_{max}=3.18; \theta=0cM$). The phenotype was mapped between markers D7S820 and GATA63F08 of chromosome 7p11.2-q32. To our knowledge we are the first to present and discuss the genotype of a patient with PAP-B of both hands only without showing PAP-A.

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Exclusion of 113 Kb of IDDM-8 interval by linkage disequilibrium analysis. *A. Davoodi-Semiromi, J-D. Shi, D. Hopkins, C-Y. Wang, J-X. She.* Department of Pathology, Immunology and Laboratory Medicine, Center for Mammalian Genetics and Diabetes Center of Excellence, College of Medicine, University of Florida, Gainesville, FL 32610, USA.

We previously analyzed 15 markers on chromosome 6q and a strong evidence of association was obtained at D6S446 using affected-sib-pair analyses in 104 Caucasian families. In this study, we attempted to narrow the interval to a small region suitable for positional cloning using new microsatellite markers and single nucleotide polymorphisms (SNPs) in 668 families with type 1 diabetes (T1D). Four new markers including two microsatellite markers and two SNPs in the IDDM 8 interval were identified. We found a significant association with one of the microsatellite marker designated D6S2724 in Caucasian populations including Americans, French and Italians. We did not, however, find a significant association when British, Mexican-American, Korean and Chinese populations were screened. The overall result for the entire data sets was very significant ($P \leq 0.00006$). We ruled out the PDCD2 gene as a candidate gene for T1D since we did not find any association when two SNPs within this gene were typed against a large number of diabetic families. In this study we excluded BAC 191N21 (113 Kb) within the IDDM-8 interval for further investigation since we were unable to find a putative transcript for the IDDM-8 gene.

Haplotype and linkage disequilibrium architecture for human cancer-associated genes. *P.E. Bonnen¹, P. Wang¹, M. Kimmel², R. Chakraborty³, D.L. Nelson¹.* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Department of Statistics, Rice University, Houston, TX, 77030; 3) Center for Genome Information, Department of Environmental Health, University of Cincinnati, Cincinnati, OH, 45267.

To facilitate association-based linkage studies we have studied the linkage disequilibrium (LD) and haplotype architecture around five genes of interest for cancer risk: ATM, BRCA1, BRCA2, RAD51, and TP53. Single nucleotide polymorphisms (SNPs) were identified and used to construct haplotypes that span 93-200 kb per locus with an average SNP density of 12 kb. These markers were genotyped in four ethnically defined populations that contained 48 each of African Americans, Asian Americans, Hispanic Americans, and European Americans. Haplotypes were inferred using an expectation maximization (EM) algorithm and the data were analyzed using D, R², Fishers exact, and the four gamete test for recombination. LD levels varied widely between loci from continuously high LD across 200 kb to a virtual absence of LD across a similar length of genome. LD structure also varied at each gene and between populations studied. This variation indicates that the success of linkage-based studies will require a precise description of LD at each locus and in each population to be studied. One striking consistency between genes was that at each locus a modest number of haplotypes present in each population accounted for a high fraction of the total number of chromosomes. We conclude that each locus has its own genomic profile with regard to LD and despite this there is the wide spread trend of relatively low haplotype diversity. As a result, a low marker density should be adequate to identify haplotypes that represent the common variation at a locus thereby decreasing costs and increasing efficacy of association studies.

SNPing OF LOR GENE IN PSORIASIS PATIENTS. *E. Giardina*¹, *F. Capon*¹, *A. Tacconelli*¹, *S. Chimenti*², *G. Zambruno*³, *G. Novelli*¹. 1) Dept Biopathology, Tor Vergata Univ, Rome, Italy; 2) Department of Dermatology, Tor Vergata Univ, Rome, Italy; 3) Molecular and Cellular Biology Unit, IDI IRCCS, Rome, Italy.

Psoriasis (OMIM 177900) is a chronic, hyper-proliferative skin disorder affecting approximately 2% of Caucasians. The disease is inherited as a complex trait and genome-wide scans have allowed to map at least 7 susceptibility loci (PSORS 1-7). We assigned the PSORS4 locus to chromosome 1q21 and have recently refined the susceptibility interval to a 100 kb segment containing the LOR gene. LOR encodes loricrin, a major keratinocyte structural protein, which is under-expressed in psoriatic lesions. We report here the genetic analysis of LOR as a PSORS4 positional candidate. We have re-sequenced the gene in 8 Italian patients carrying disease associated alleles at two PSORS4 microsatellite loci. This analysis identified 1 SNP in the promoter region, a non-conservative substitution and a 6bp in-frame duplication in exon 2. Typing of 90 trios demonstrated that all three variants are common in the Italian population, the frequency of minor alleles being > 0.25. An assessment of Hardy-Weinberg equilibrium (HWE) among patients' chromosomes identified a significant heterozygous excess ($p = 1.8 \times 10^{-4}$) for the exon 2 SNP. The analysis of 40 unrelated healthy controls confirmed that this SNP is in equilibrium among unaffected, indicating that the deviation from HWE observed in patients is likely to be disease-related. Work funded by the Italian Ministry of Health.

A comparative study on the block structure of linkage disequilibrium in the Japanese and Korean populations. *T. Akesaka*¹, *K. Song*², *J. Ohashi*¹, *S.G. Lee*², *K. Tokunaga*¹. 1) Department of Human Genetics, University of Tokyo, Tokyo, Japan; 2) Department of Biochemistry, University of Ulsan College of Medicine, Seoul, Korea.

The extent of linkage disequilibrium (LD) in human genome provides the important information for the identification of variations which predispose to human diseases. However, the strength of LD may differ among human populations as well as chromosomal regions. We investigated LDs and haplotypes defined by SNPs in chromosome 1p36.2 region in the Japanese and Korean populations. Forty SNPs in 300 kb region encompassing from the tumor necrosis factor receptor 2 (TNFR2: TNFRSF1B) gene to the procollagen-lysine, 2-oxoglutarate 5-dioxygenase (PLOD) gene were examined by means of fluorescence correlation spectroscopy combined with the PCR-sequence specific primer method (PCR-SSP-FCS) in 200 healthy Japanese and by direct sequencing in 96 healthy Koreans. Analysis of LDs between two SNP pairs demonstrated clear contrasts in LD strength among different regions. Interestingly enough, a long range block with strong LDs of approximately 80kb including three genes was found in both Japanese and Koreans. And three common haplotypes composed of more than 20 SNPs were observed from the block in both populations. However, in the regions around TNFR2 and CD30 (TNFRSF8) genes, significant LD was observed only within the genes. LD strength and haplotype frequencies were similar between Japanese and Koreans, probably because of strong genetic affinities between the two populations.

Estimation of haplotype frequencies and diplotype configuration for each subject using pooled DNA data. *T. Ito*^{1,2}, *S. Chiku*³, *E. Inoue*^{1,4}, *M. Tomita*¹, *N. Kamatani*^{5,6}. 1) Gene Discovery Team, Japan Biological Information Research Center, Tokyo, Japan; 2) Mitsubishi Research Institute, Inc., Tokyo, Japan; 3) Fuji Research Institute Corporation, Tokyo, Japan; 4) NEC Corporation, Tokyo, Japan; 5) Division of Statistical Genetics, Institute of Rheumatology, Tokyo Women's Medical University, Tokyo, Japan; 6) Institute of Rheumatology, Tokyo Women's Medical University, Tokyo, Japan.

Haplotype inference is important for many genetic approaches including the process of assigning a phenotype to a genetic region. Usually the frequencies of haplotypes as well as diplotype configuration of each subject are estimated from a set of genotypes of the subjects in a population. We have developed an algorithm to infer haplotype frequencies from pooled DNA data. The input data are the genotypes in pooled DNA samples each of which contains the quantitative genotype data from 1-10 subjects. The algorithm infers by the maximum likelihood method both frequencies of the haplotypes in the population as well as sets of diplotype configurations in the pooled samples by expectation-maximization (EM) algorithm. The algorithm was implemented in the computer program "ldpooled". Using this program, the published genotype data for SAA gene as well as smootheline gene were analyzed. Our study has shown that the haplotype frequencies in a population can be inferred although with some limitations from the pooled DNA data by the maximum likelihood method. Our present method may be useful when the genotyping is performed on pooled DNA.

SNP analysis and linkage disequilibrium map of the human chromosome 22 using Arrayed Primer EXTension (APEX) technology. *E. Lohmussaar*^{1,2}, *J. Zernant*², *R. Mägi*³, *T. Puurand*², *N. Tõnisson*², *I. Valvas*², *G. Slavin*², *A. Kurg*¹, *M. Remm*^{1,3}, *A. Metspalu*^{1,2}. 1) IMCB University of Tartu/Estonian Biocentre; 2) Asper Ltd. Tartu, Estonia; 3) Biodata Ltd. Tartu, Estonia.

One of the goals of the human genome project is to further understand the genetic basis of human variation. DNA sequence variants across the genome are responsible for a variety of phenotypes such as disease risk or variable drug response. The whole-genome linkage disequilibrium mapping and haplogroup description are the prerequisites for genome wide association studies. Using the completed sequence of human chromosome 22 and the extensive set of genetic variants across 22q, we have performed genotyping of Estonian and German samples and CEPH families with 1279 SNP markers (at a median spacing of 35 kb). An array with 5200 oligonucleotides was designed to genotype each SNP twice from both DNA strands simultaneously using APEX technology. Allele frequencies, Hardy-Weinberg equilibrium data and heterozygosities were calculated for each genotyped marker. We have characterized the patterns of linkage disequilibrium and calculated D for the whole 33Mb of chromosome 22q. Our results show several areas of the chromosome with very high level of LD, notably at positions 11-16 Mb and 21-27 Mb of the reference sequence. We also estimated the average extent of useful LD ($D > 0.5$) and similar results were obtained in analysis of separate populations of unrelated individuals. It appears that the major problem was the low minor allele frequency (less than 10%), which means that the quality of SNPs in current public databases is an important issue and will need additional testing in order to find out useful SNPs in regarding to genotyping technology. Construction of LD maps across the human genome and identifying haplotypes in individual genomic regions will facilitate the identification and characterization of genetic variants responsible for common complex diseases.

Linkage disequilibrium at the dopamine transporter locus (*SLC6A3*). A.M. Kang^{1,2}, A.J. Pakstis², K.K. Kidd². 1) Dept Linguistics, Brain & Language Track; 2) Dept Genetics, Yale U, New Haven, CT.

The dopamine transporter (DAT; locus *SLC6A3*) is implicated in disorders like Parkinson's Disease, substance abuse problems, attention-deficit hyperactivity disorder (ADHD), and bipolar disorder. Most early studies used a case-control association design with a single 3'-UTR VNTR, which likely was not testing the entire locus because of its ~50-kb length. Elucidating the linkage disequilibrium (LD) pattern at this telomeric locus is important to determine the appropriate number and spacing of polymorphisms needed for haplotype association studies.

We report allele frequencies in 38 worldwide populations for seven single nucleotide polymorphisms (SNPs) and two variable number tandem repeats (VNTRs) spanning the entire ~50 kb locus. Preliminary LD analyses including only the five most heterozygous SNPs on 35 populations demonstrate consistently low overall disequilibrium across all geographic regions, as measured by r^2 ; this pattern differs from that observed at *DRD2* and *PAH* from similar studies in our lab. F_{ST} calculations for the SNPs show a particularly high value of .37 for an intron 9 SNP, compared to a reference distribution obtained in our lab from 94 biallelic sites (mean = .14, s.d. = .07), possibly suggesting some selective pressure at or around this site.

Pairwise LD across smaller segments was quite variable among, and even within, geographic regions. A 15-kb segment spanning the 5' third of the locus (promoter to intron 4) showed little disequilibrium in populations from Africa, SW Asia, Europe, and much of east Asia. Even three of the four North American populations had little LD. A 20-kb segment from intron 4 to intron 9, representing the middle third of the locus, showed no LD in any European or east Asian population but high levels in some African and SW Asian populations. The 3' end (~16 kb) spanning intron 9 to the 3'-UTR showed high LD in many SW Asian and European populations but not in other geographic regions. These results suggest ~16-kb spacing of SNPs may not be sufficiently dense coverage at this locus. [Supported in part by NIH grants GM57672 and MH62495].

Analysis of a strong candidate gene for late onset Alzheimer's disease within the 1-lod interval on chromosome

10. *P. Nowotny¹, A. Myers¹, A. Mander³, H. Marshall¹, P. Holmans³, R. Crook⁴, S. Smemo¹, M. Dunston⁵, M. Hamshere⁵, M. O'Donovan⁵, L. Jones⁵, J. Williams⁵, M.J. Owen⁵, J. Hardy⁴, A. Goate^{1,2}.* 1) Dept Psychiatry, Washington Univ Sch Medicine, St Louis, MO; 2) Department of Genetics, Washington University School of Medicine, St. Louis, MO, United States; 3) MRC Biostatistics Unit, Cambridge, United Kingdom; 4) Department of Neuroscience, Mayo Clinic Jacksonville, FL, United States; 5) Department of Psychological Medicine, Cardiff, United Kingdom.

We performed a two-stage genome screen to search for novel risk factors for late onset Alzheimer's disease (AD). In the first stage, 16 regions gave a multipoint lod score greater than 1. In the second stage we followed up in these regions with more samples and markers. The strongest evidence for linkage in the second stage was on chromosome 10, where we obtained a peak multipoint LOD score (MLS) of 3.9. Urokinase-plasminogen activator (PLAU), a gene putatively involved in the degradation of β -amyloid, the toxic fragment of APP, maps within our linkage peak on chromosome 10. We have analyzed 12 polymorphisms within this gene, including three coding variants. None of the coding variants were significantly associated with risk for LOAD in the case-control series we examined. We also found no significant frequency differences in seven additional non-coding SNPs. The most common haplotype formed from three non-coding polymorphisms (2 intronic and one synonymous change) spanning exons 8 and 9 of PLAU has a lower risk for LOAD than other haplotypes. The haplotype does not appear to account for our linkage results on chromosome 10. There are two possible explanations for these results: that there is variation elsewhere in the PLAU gene or in a neighboring gene in linkage disequilibrium with the SNPs genotyped in this study that influences risk for AD or alternatively this result could represent type I error. We are currently genotyping additional SNPs in a 50kb region flanking the PLAU gene to evaluate these alternatives.

APOE promoter region is associated with age at onset of Amyotrophic Lateral Sclerosis. *Y-J. Li¹, M.A. Pericak-Vance¹, J.L. Haines⁴, N. Siddique², D. McKenna-Yasek³, E.R. Martin¹, W-Y. Hung², P. Sapp³, C.I. Allen¹, W. Chen², B. Hosler³, A.M. Saunders¹, L. Dellefave², R.H. Brown Jr.³, T. Siddique².* 1) Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Dep't Neurology, Northwestern Univ Med School, Chicago IL; 3) Day Neuromuscular Lab, Mass Gen Hosp, Boston, MA; 4) Program in Human Genetics, Vanderbilt Univ, Nashville, TN.

Amyotrophic lateral sclerosis (ALS) is a progressive neurologic disorder with midlife onset and a rapid downward course of paralysis ending in death. Several studies have investigated the relationship between APOE and ALS but generally small datasets were used and the conclusions were inconsistent. We investigated APOE and its promoter region in 461 families with at least 1 case of ALS. We genotyped 5 SNPs in addition to the APOE functional polymorphism. The designation of each SNP is based on its distance in basepairs from the beginning of exon1. SNP-491AT, SNP-427CT, and SNP-219GT are located in the APOE promoter region, SNP+113CG in intron1, and SNP+5361CT in APOE 3' region. The PDT and TRANSMIT (TRM) programs were used to test markers for linkage and association with ALS. By using the full dataset (461 families), no markers demonstrated significant association to ALS. We also examined a possible role of APOE on age at onset (AAO) by stratifying based on the AAO of affecteds. The AAO in our ALS dataset ranged from 15 to 81 years old. For the PDT and TRM tests we found that SNP-219GT ($p=0.04$, 0.03 , respectively) and SNP+5361CT ($p=0.01$, 0.04 , respectively) showed significant association to ALS in the families with $AAO \geq 50$ (165 families). To further test this age related hypothesis, we treated AAO as a quantitative trait and performed association analysis in the full dataset. The TDTQ5 method implemented in QTDT program was employed. SNP-219GT and SNP+113CG showed evidence of association to AAO (both $p=0.03$), as did the APOE functional polymorphism ($p=0.002$, with the APOE2 allele protecting against earlier onset). Our findings suggest that APOE may express its strongest effect on the risk of ALS through AAO rather than on the risk alone.

A complete ascertainment haplotyping in a SNP-intensive gene: Clear population-specific haplotypes in the Cardiac Ankyrin Repeat Protein (CARP) gene. *B.T. Harmon, J.M. Devaney, E.P. Hoffman.* Research Center for Genetic Medicine, 111 Michigan Ave., NW Washington, DC 20010.

The response to exercise varies widely between individuals and is considerably influenced by genetic variables. We have a SNP association study underway where 1,400 young adults are exercised to quantitate 4 phenotypes (entry strength, entry muscle size by MRI, percentage change in strength after 12 wks supervised resistance exercise, percentage change in size) (Factors Affecting Muscle Size and Strength; FAMuSS). In parallel, high throughput and highly sensitive SNP screening is being pursued, with genotyping of ethnically diverse individuals to determine SNPs predisposing to muscle size and strength. One gene strongly implicated in the response of muscle and heart to exercise is the cardiac ankyrin repeat protein (CARP) gene. The CARP gene was screened for SNPs in DNA from 96 individuals representing a variety of different ethnic backgrounds. SNPs were detected by direct sequencing of coding sequences, exon/intron boundaries, and the 5 and 3 UTRs using ABI 3100 automated sequencers. We identified 10 novel SNPs and 1 deletion in the CARP gene, none of which were identified by existing SNP resource databases. We used Clark's haplotype subtraction algorithm to resolve the SNPs into haplotypes, many of which were population-specific. Of the 10 SNPs found, 3 changed an amino acid, and 4 were found to be private SNPs that were present in only a single individual of the 96 studied. This analysis shows that a gene that is highly responsive to the physiological variable of exercise is a hot spot for SNPs.

Association of angiotensin converting enzyme (ACE) gene with hypertension and ACE level in Nigerians. Y.

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Angiotensin converting enzyme (ACE) gene has been reported to be associated with cardiovascular disease (CVD). Hypertension is an important risk factor in CVD. To identify the susceptibility SNPs of the ACE gene, 829 Nigerians from 207 families were genotyped at 32 single nucleotide polymorphisms (SNPs) within the ACE gene. 300 unrelated individuals are included in the first haplotype inference using PHASE. The SNPs were then grouped into 6 blocks such that pairwise linkage disequilibrium were strong ($D' \geq 0.8$) within each block. Association of hypertension and ACE level with ACE haplotypes were tested within each block using TRANSMIT. We identified two haplotypes in two blocks that were associated with hypertension. TCCA (ace1 ace2 ace3 ace4_2400) (freq=4.6%) global p=0.0071; ADGAAGG (newace6_8578 aceI_D_1448_14488_14521 ace8_14521_15214)(freq=28.4%) global p=0.0933. This haplotype (ADGAAGG) is also significantly associated with systolic blood pressure (p=0.0017) using regression model under dominant mode of inheritance after adjusting sex and age. Significant association of ACE haplotypes with ACE level were identified in two blocks. CCCT (29%) (ace1 ace2 ace3 ace4_2400) global p=0.0021; ATTCCA (6.9%), ACTCCG (42.7%), and ACTTCG (26.1%) (_15444_19942_20058_20120_20326_20397) with global p=0.0032. Our results suggest possible relationship of these haplotypes (blocks) with hypertension.

The efficacy of using haplotypes to assay common variation in genes. *M. Loomer^{1,2}, S.B. Gabriel^{1,2}, J. Platko^{1,2}, C. O'Donnell^{2,3}, M.J. Daly¹, E.S. Lander^{1,2,4}, J.N. Hirschhorn^{1,2,5,6}.* 1) Center for Genome Research, Whitehead Institute, Cambridge, MA; 2) Cardiogenomics PGA, NHLBI; 3) NHLBI Framingham Heart Study, Framingham, MA; 4) Biology, MIT, Cambridge, MA; 5) Genetics, Harvard Med. School, Boston, MA; 6) Genetics and Endocrinology, Children's Hospital, Boston, MA.

Surveys of random genomic regions have shown that the genome contains large "blocks" of linkage disequilibrium; these blocks contain a few common haplotypes that are highly correlated with publicly available common SNPs in the block (Gabriel et al., Science 2002). We surveyed haplotype patterns in genes and tested whether common haplotypes in genes are correlated not only with publicly available SNPs but also with common SNPs discovered by directed resequencing.

For analysis, we chose 33 genes, all candidates for regulating cardiovascular traits. Using TSC and BAC overlap SNPs, we defined blocks. We identified additional SNPs within these blocks by resequencing exons and nearby introns in a multiethnic panel of 40 individuals. Within the blocks, we defined a "framework" of common haplotypes in a sample of 92 Caucasian individuals from 12 multigenerational pedigrees (96 independent chromosomes). We also analyzed the relationship between block boundaries and gene landmarks.

To date, we have genotyped enough common SNPs to analyze 22 of the 33 genes, covering 1.1 Mb of genomic DNA. We successfully genotyped 353 SNPs in these genes, of which 274 were polymorphic and 243 had minor allele frequencies >5%. Haplotype blocks defined by these SNPs averaged between 20 and 25 kb in length (covering 814 kb), and there were generally 3-6 common haplotypes per block, consistent with earlier results from random genomic regions. 27 SNPs identified by resequencing had minor allele frequencies >5% and resided within the haplotype blocks defined by our framework. Of these 27 SNPs, only 5 defined new haplotypes that were not already present within the framework. We conclude that common haplotypes within blocks of linkage disequilibrium are likely to capture most of the common variation within their span.

A NEW CROHN'S DISEASE PREDISPOSING *CARD15* HAPLOTYPE IN ASHKENAZI JEWS. *K. Sugimura*^{1,2}, *K.D. Taylor*¹, *Y-C. Lin*¹, *T. Hang*¹, *D. Wang*¹, *Y-M. Tang*¹, *N. Fischel-Ghodsian*¹, *S.R. Targan*², *J.I. Rotter*¹, *H. Yang*¹. 1) Medical Genetics; 2) IBD Center Res. Lab.; Cedars-Sinai Medical Center, Los Angeles, CA.

Aim: Crohn's disease (CD) is an inflammatory bowel disease (IBD) that occurs with a 2-3 fold higher frequency in Jews. *CARD15* was identified to be the IBD1 gene that is located on chr.16q12 and showed significant linkage to CD by multiple groups. Three *CARD15* coding variants (R675W, G881R, and 980fs) are associated with CD individually. Since more than 70% of CD patients do not have any of these major *CARD15* disease-predisposing mutations (DPMs), the aim of this study was to determine if additional DPMs exist at the IBD1 locus in the high-risk Jewish group.

Methods: Six microsatellite markers spanning IBD1 (34cM) were genotyped in 28 Jewish CD families and two-point linkage analysis using SIBPAL from SAGE was performed. SNPs in *CARD15* (R675W, G881R, 980fs; plus a *CARD15* background marker S241P and a new SNP as described below) were genotyped in the families and in a Jewish case control panel (114 CD, 80 controls) using TaqMan MGB. **Results:** Significant linkage of IBD1 to CD in Jews remained after excluding the 3 major *CARD15* DPMs (mean allele sharing, MAS, 0.47 to 0.70 with a peak at D16S403, $p = 0.0008$). These data suggested the existence of additional predisposing genes at the IBD1 locus or additional *CARD15* DPM(s) in Jews. Furthermore, in the Jewish case-control sample, the S241P background haplotype without any of the three known DPMs was significantly associated with CD (OR=3.13, $p=0.0023$). This suggested the existence of unrecognized DPMs on a S241P haplotype in Jews. Sequencing of *CARD15* translated regions from Jewish patients with ($n=7$) and without ($n=5$) the S241P haplotype identified one new variant (IVS8+158, JW1). The S241P-JW1 combination defined a more specific haplotype associated with an even further increased risk for CD in Jews (OR=5.75 $p=0.0005$) than that provided by S241P alone. **Conclusion:** These results suggest that, in Ashkenazi Jews, other predisposing genes in the IBD1 locus or other DPMs on the *CARD15* S241P-JW1 haplotype may lead to CD in this high risk group.

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Linkage disequilibrium mapping using genotype data from unrelated individuals. *T. Wang*¹, *H. Jacob*². 1) Division of Biostatistics; 2) Human and Molecular Genetic Center, Medical College of Wisconsin, Milwaukee, WI.

Linkage disequilibrium (LD) approach has been successfully used for fine mapping of disease genes. Reconstruction of haplotypes are essential in most of current LD studies. We develop a multipoint LD method to estimate joint genotypic frequencies of a QTL and multiple markers using observed phenotypic trait values and marker genotypes directly. A summarized statistic is proposed as an overall association measure between the QTL and markers. Detection of the QTL position can then be performed based on this association pattern. A permutation procedure is also implemented for testing of the association.

Sample size estimates for candidate gene case-control studies in pharmacogenetics. *L.J. Sheffield.* Genetic Health Services Victoria, Murdoch Childrens Research Institute, University of Melbourne, Melbourne, Victoria, Australia.

Mapping studies using linkage disequilibrium methods frequently use the TDT or case-control design. The case-control design is statistically more powerful but may be prone to bias due to population sub-stratification. It is not always possible to genotype parents in pharmacogenetic studies and so case-control designs are often used. Population sub-stratification can be controlled for by matching controls with cases for variables like ethnic group. This presentation presents details of sample size estimates for case-control studies of candidate genes for disease or drug effect (pharmacogenetics). There are some newly described methods for sample size calculation for case-control studies when whole genome wide linkage disequilibrium studies are planned. It has been suggested that these studies require 100,000-500,000 polymorphisms. At present very few such studies are performed because of the cost of typing so many markers. Most studies being performed today are either of candidate genes or candidate gene regions of a particular chromosome. As such they do not require the stringent Bonferroni correction that whole genome scans do. I suggest a correction for 50 independent tests for candidate gene studies. I present sample size estimates for candidate gene case-control studies for the first time with different marker frequencies. It should be noted that until recently the only method available for estimating such sample sizes was the classical statistical approach which does not assume any genetic model. A comparison is made with this approach which both over estimates and underestimates the sample size for the dominant model which it is closest in concept to the genetic methods. For example for an odds ratio of 3 (Type I error of .001, power of 80%) the classical method suggests 148 cases and controls need studying whilst the genetic methods estimate 132 of each for the dominant model and 219 for the additive model. As the known genotypic relative risk (for each genotype) may be able to be estimated from previous work the genetic methods should be used.

Genome-wide distribution of linkage disequilibrium in the Samoan population. *H.-J. Tsai*¹, *G. Sun*², *D. Smelser*², *D.E. Weeks*^{1, 4}, *S. McGarvey*³, *R. Deka*². 1) Human Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Environmental Health, University of Cincinnati, Cincinnati, OH; 3) International Health Institute, Brown University, Providence, RI; 4) Biostatistics, University of Pittsburgh, Pittsburgh, PA.

Recently, whole genome scanning for susceptibility loci based on linkage disequilibrium (LD) has been proposed as a powerful strategy for mapping common complex diseases, especially in isolated populations. In this study, we have recruited 176 families from US territory of American Samoa, and 96 unrelated controls from American Samoa and the independent country of Samoa. We have examined the distribution of LD in the family, control and combined data separately by using 382 autosomal microsatellite markers across the whole genome. We have tested the relationship between adjusted multiallelic disequilibrium and recombination by fitting a regression model. We have obtained a slope of -0.022 (SE = 0.006, P = 0.00052) for family data, and a slope of -0.022 (SE = 0.004, P < 0.00001) for combined data. Based on our results, LD decays steadily as recombination between autosomal markers increases in the Samoan population. These results imply that it may be feasible to do genome-wide LD mapping in the Samoans. Supported by NIH grants AG09375, HL52611, DK55406, and DK59642.

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Detecting Linkage Disequilibrium between Genes. *B.A. Salisbury, J.C. Stephens, A. Windemuth, J.A. Schneider, R.S. Judson.* Genaissance Pharmaceuticals, New Haven, CT.

Techniques for detecting and measuring Linkage Disequilibrium (LD) between complex loci such as genes are in their infancy. We have explored several new approaches empirically using data acquired by sequencing functional regions of genes. A special emphasis was placed on contrasting SNP-based and haplotype-based (whole-gene) LD tests. We considered the effects of sample size, population structure, and physical distance on test power and LD strength. All factors we examined were important. Of the dozens of genes we looked at, most were drawn from Chromosome 19, including an apolipoprotein gene region with remarkably variable patterns of LD. We sampled as deeply as several hundred unrelated individuals, who represented broad ethnic diversity.

Molecular Screen of Retinoid-Related Orphan Receptor Gamma as a Positional Candidate Gene for Type 2 Diabetes. *H. Wang, WS. Chu, SC. Elbein.* Dept Endocrinology, Central Arkansas Veterans healthcare System and University of Arkansas for Medical Science, Little Rock, AR.

The retinoid-related orphan receptors ROR alpha, beta, and gamma constitute a subfamily of nuclear orphan receptors. ROR gamma (RORC) is induced during adipocyte differentiation and functions as an active transcription factor to induce the expression of PPAR gamma. Thus, RORC is a candidate gene for diabetes pathogenesis. Furthermore, RORC is located on chromosome 1q22, a region that has been linked to type 2 diabetes in Utah, French and English Caucasians, Pima Indians, and Old Order Amish. To test the hypothesis that mutations of RORC account for part of this linkage, we screened 11 exons of the RORC gene for variants, including 1-kb upstream, intronic regions flanking the exons, and the entire 3 untranslated region (UTR), spanning approximately 25 kb. We used SSCP analysis under 2 conditions with bi-directional fluorescent sequence confirmation to screen 32 subjects including 16 Utah Caucasian individuals from families showing linkage to chromosome 1 and 16 diabetic African American individuals. We detected 11 single nucleotide polymorphisms (SNPs), ranging from the promoter region to intron 10. Only 1 SNP was nonsynonymous, resulting in Ala to Gly at residue 464 (exon 10). All other SNPs were noncoding. One SNP (intron 3) was unique to Caucasians, and three SNPs (A464G, intron 2, intron 6) were specific to African American subjects. We initially typed 6 SNPs spanning the gene from the promoter to 3 UTR in 131 unrelated Utah Caucasian cases (61 members of familial T2DM kindreds) and 118 ethnically matched controls. Two SNPs (intron 8, A464G) were very rare or absent in Caucasians and not analyzed. Allele frequencies for the remaining 4 SNPs did not differ between cases and controls ($p > 0.2$). Three of the 4 SNPs were in strong linkage disequilibrium ($D > 0.85$), but the single SNP at the most 3 end of the gene showed no significant linkage disequilibrium with the more 5 SNPs. Although available data suggest that RORC cannot explain the linkage of T2DM in this region, additional typing is in progress, and work is also in progress to test for metabolic abnormalities.

The effect of LD structure on genotype-phenotype relationships at the *DBH* locus. C.P. Zabetian^{1,2}, S.G. Buxbaum³, R.C. Elston³, M.D. Kohnke⁴, G.M. Anderson⁵, J. Gelernter^{6,7}, J.F. Cubells^{6,7}. 1) Dept of Neurology, Univ of Washington, Seattle, WA; 2) Dept of Neurology, VAPSHCS, Seattle, WA; 3) Dept of Epidemiology and Biostat, Case Western Reserve Univ, Cleveland, OH; 4) Univ Hospital of Psychiatry and Psychotherapy, Tuebingen, Germany; 5) Child Study Ctr, Yale Univ, New Haven, CT; 6) Dept of Psychiatry, Yale Univ, New Haven, CT; 7) Dept of Psychiatry, VACHS, West Haven, CT.

Advances in technology, including the growing public database of some 2.7 million candidate single nucleotide polymorphisms (SNPs), promise to make genome-wide linkage disequilibrium (LD) mapping of loci influencing complex traits feasible in the near future. Recent studies have suggested that the extent of LD throughout the genome is extremely variable even at intragenic scales, and that the genome might be divided into discrete 10-100 kb "blocks" of LD. Whether such blocks of LD, defined statistically based on common SNPs, will be useful for detecting associations with genes underlying complex traits is presently unknown, and few studies have examined the effect of LD on the association of specific markers to traits using real data. The *DBH* gene, which encodes dopamine beta-hydroxylase (DBH) and is the major quantitative trait locus for plasma DBH activity, is well suited as a simple model to address this question. We recently demonstrated that a single putative functional SNP (-1021C®T) in the *DBH* promoter accounted for 35-52% of the total variation in plasma activity levels in 3 distinct populations. Here, we present evidence that in general, LD extended over short distances at the *DBH* locus. The extent of LD between -1021C®T and 11 surrounding biallelic markers strongly influenced the strength of association of each marker to plasma DBH activity. This relationship was better captured by measures of LD that are relatively dependent on allele frequencies, such as d^2 . The most distant markers in the 5' upstream region and 3' half of the gene were not significantly associated with DBH activity. These findings provide further evidence that a thorough understanding of LD structure will be necessary prior to designing genome-wide LD mapping studies.

Linkage disequilibrium in the *NIDDM1* and *CYP19* regions of chromosomes 2 and 15. *J.K. Wittke, I. Yoshiuchi, H. Mashima, C. Roe, N.J. Cox.* Dept. of Human Genetics, The University of Chicago, Chicago, IL.

Previous studies mapped susceptibility loci for type 2 diabetes (T2D) to the *NIDDM1* region of chromosome 2 (Hanis et al, Nat Genet 1996; Horikawa et al, Nat Genet 2001) and the *CYP19* region of chromosome 15 (Cox et al, Nat Genet 1999) using data from a genome screen on Mexican Americans (MA) from Starr County, TX. Linkage disequilibrium (LD) among SNPs typed for the follow-up studies conducted in these regions reveal differences in the extent of LD. We used all SNPs with a minor allele frequency >0.1 (59 on chromosome 2 and 47 on chromosome 15), and calculated D' for SNP pairs using haplotype frequencies estimated with an EM algorithm. The same subjects (108 MA patients with T2D and a random sample of 112 MA, all unrelated) were typed for both the chromosome 2 and the chromosome 15 SNPs. In the random sample, the proportion of marker pairs on chromosome 15 with $D'>0.9$ was significantly greater than on chromosome 2 to a distance of 55kb (where 4% of pairs have $D'>0.9$ on chromosome 2). The proportion of marker pairs with $D'>0.33$ was significantly greater for chromosome 15 than for chromosome 2 to a distance of 120 kb. We also found more haplotype diversity across the *NIDDM1* region than across the *CYP19* region, and both regions show more haplotype diversity than was reported for markers across the 5q region in a different population (Daly et al, Nat Genet 2001). Finally, although overall patterns of LD were similar in the patient and random samples for both the chromosome 2 and chromosome 15 markers, a higher proportion of marker pairs showed extensive LD in the patient sample in the *NIDDM1* region than in the random sample. These differences in LD are apparent only for markers less than 20 kb apart and are largely attributable to higher LD among SNPs in the *CAPN10* gene in the patient sample relative to the random sample. The differences in LD between the *NIDDM1* and *CYP19* regions suggests that it will be more difficult to detect association of genetic variation with disease in the *NIDDM1* region than in the *CYP19* region, because LD extends over a shorter distance. Lesser LD in the *NIDDM1* region will also lead to more tightly localized disease associations.

A Tobit Variance-Component Method for Analyzing Truncated Trait Data. *M.P. Epstein, X. Lin, M. Boehnke.*
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Variance-component methods are flexible and powerful procedures for mapping genes that influence quantitative traits. However, variance-component methods make the critical assumption that the quantitative trait data within a family either follows, or can be transformed to follow, a multivariate normal distribution. Violation of the multivariate normality assumption can occur if trait data are truncated at some threshold value. Trait truncation can arise in a variety of ways, including assay detection limitation or confounding due to medication.

Valid inference of truncated data in linkage analysis requires the development of a modified variance-component method that directly models the truncation. We present such a method, which we call the tobit variance-component method. Using simulated data, we compare and contrast the characteristics of the traditional and tobit variance-component methods for linkage analysis of truncated trait data. Our results suggest that (1) analyses of truncated data using the traditional variance-component method leads to severe parameter underestimation and a modest increase in false positive linkage findings, while (2) analyses with the tobit variance-component method lead to unbiased parameter estimates and type I error for linkage tests that is slightly conservative.

As an example, we simulated latent trait data with overall genetic heritability of 0.75 for 400 sibtrios and truncated the lower 25% of the data. Analyzing the truncated data with the traditional method yielded empirical type I error rates of .0663 and .0183 for nominal $p=0.05$ and 0.01, respectively. Also, mean parameter estimates were only 55%-70% of the true simulating values. Analyzing the same data using the tobit method yielded empirical type I error rates of .0503 and .0094, respectively, and unbiased parameter estimates.

Genetic mapping of two loci for familial hypercholanemia in the Amish. V. Carlton¹, E. Puffenberger², A. Knisely³, B. Shneider⁴, D. Morton², G. Salen⁵, L. Bull¹. 1) University of California, San Francisco, CA; 2) Clinic for Special Children, Strasburg, PA; 3) King's College Hospital, London UK; 4) Mount Sinai School of Medicine, NY; 5) UMDNJ-New Jersey Medical School, East Orange, NJ.

Familial hypercholanemia (FHC) is a rare childhood disorder characterized by elevated serum concentrations of bile acids and nutritional malabsorption. To study the genetic basis of FHC, we performed a 5 cM whole genome screen on Amish patients. We initially used a conservative approach by considering affected only those 5 patients who met strict criteria (definite FHC); similarly, but less severely affected patients were classified as possible FHC. Genome screen data were analyzed using a linkage disequilibrium approach by searching for shared haplotypes in the definite FHC patients. Three of the 5 definite FHC patients were homozygous for a shared haplotype (a statistically significant finding). The region was genotyped in 9 additional possible FHC patients (4 from families included in the genome screen and 1 each from 5 additional families), 6 of whom were found to be homozygous for the haplotype identified in the genome screen. We infer the presence of an FHC locus in this region, which we term *fhc1*. A candidate gene in this region was sequenced and found to contain a putative mutation not seen on 190 Caucasian control chromosomes; this gene's product is associated with tight junctions. Several unaffected siblings of children with FHC are also homozygous for the disease-associated change. We infer that this mutation in the candidate gene for *fhc1* exhibits incomplete penetrance. Several Amish patients do not share the *fhc1* disease-associated haplotype. Further study of these patients identified a second shared haplotype elsewhere in the genome, pointing to a second FHC locus, *fhc2*. A disease associated haplotype in the *fhc2* region is shared homozygously by 3 affected siblings and one patient in another family. A candidate gene in this region was sequenced and found to contain a putative mutation not seen on 182 Caucasian control chromosomes. To our surprise, one Amish patient does not share the *fhc1* or *fhc2* disease associated haplotypes. We infer that a third FHC locus exists in the Amish.

ADHD: DRD4 Linkage analysis in extended pedigrees. *M. Arcos-Burgos¹, D. konecki¹, D.A. Pineda², F. Lopera², J.D. Palcio², K. Berg¹, X. Castellanos³, J. Bailey-Wilson¹, M. Muenke¹.* 1) NHGRI, NIH, Bethesda, MD; 2) University of Antioquia, Colombia; 3) NYU Child Study Center, NY.

Despite several linkage and linkage disequilibrium studies showing significant positive association/linkage, the co-segregation of DRD4 mutations with ADHD has not yet been found. Extended pedigrees can be a powerful and feasible method to determine co-segregation of polymorphisms with a trait. Here we are presenting a candidate gene approach for DRD4 by using 15 extended families coming from a genetic isolate, segregating ADHD. We genotype two intragenic DRD4 polymorphisms and 4 surrounding STR markers (D11S4177-D11S4046-D11S4146-D11S1323). Parametric and non-parametric linkage analyses assuming or not heterogeneity were performed. The two point results are reported in Table 1. A review of each family showed that 4 families presented results that are significantly suggestive of linkage. We had a very low value of informativeness to the DRD4 intragenic markers and therefore other markers will be necessary in screening the gene.

Marker	LOD_score	a HLOD	NPL_score	p-value
D11S4177	1.66	1.0, 1.67	1.44	0.08
D11S4046	1.25	1.0, 1.25	1.54	0.07
PromDRD4	-5.28	0.0, 0.0	-0.62	0.72
VNTR-DRD4	-1.36	0.2, 0.03	0.52	0.27
D11S4146	-3.25	0.3, 0.83	1.07	0.13
D11S1323	-3.40	0.3, 0.8	1.06	0.13

Mapping of a new autosomal dominant non-syndromic hearing loss (DFNA43) to chromosome 2q12. *E. Flex*^{1,2}, *M. Mangino*^{1,2}, *M. Mazzoli*³, *A. Martini*⁴, *A. Pizzuti*^{1,2}, *B. Dallapiccola*^{1,2}. 1) IRCCS S. Giovanni Rotondo CSS-Mendel Institute, Rome, Italy; 2) Department of Experimental Medicine and Pathology, University of Rome La Sapienza, Rome, Italy; 3) U.O.A. Otorinolaringoiatria Az. Ospedaliera di Padova Padova, Italy; 4) Department of Audiology, University of Ferrara, Ferrara, Italy.

Hearing impairment (HI) is the most frequent sensory defect characterised by a large genetic heterogeneity. Approximately 80% of genetic hearing losses are non-syndromic and 15-25% of them exhibit an autosomal dominant inheritance. We analysed an Italian three-generation family in which a non-syndromic hearing impairment is transmitted as an autosomal dominant trait. Onset of HI in all affected subjects occurred in the second decade of life with subsequent gradual progression from moderate to profound loss. HI was bilateral and symmetrical, involving all frequencies. After excluding known DFNA loci using markers, listed on the Hereditary Hearing Loss Homepage (URL: <http://dnalab-www.uia.ac.be/dnalab/hhh>), a genome wide scan was conducted with 358 highly informative microsatellite markers using the ABI PRISM Linkage Mapping Set (PE Applied Biosistem, USA). Significant linkage ($Z_{max}=4.21$; $q=0$) was obtained with markers of chromosome 2p12. This result was confirmed by multipoint analysis ($Z_{max}=4.51$), using the location score method. Haplotype analysis defined a 9.6 cM disease-gene interval, non overlapping with all loci identified on chromosome 2. Fine mapping and identification of candidate genes are in progress. Work supported by Italian Ministry of Health.

Genetic mapping of Cobalamin C deficiency: putative linkage to 1q. J.L. Atkinson^{1, 2}, A. Paterson^{1, 3}, D. Renaud⁴, J.T.R. Clarke^{5, 6}, B. Wilcken⁷, D. Bulman⁸, J.M. Rommens^{1, 2}. 1) Program in Genetics & Genomic Biology, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Departments of Molecular & Medical Genetics, University of Toronto, Toronto, Ontario, Canada; 3) Public Health Sciences, University of Toronto, Toronto, Ontario, Canada; 4) Division of Child and Adolescent Neurology, Mayo Clinic, Rochester, MN; 5) Clinical & Metabolic Genetics, The Hospital for Sick Children, Toronto, Ontario, Canada; 6) Pediatrics, University of Toronto, Toronto, Ontario, Canada; 7) Biochemical Genetics Service, The Children's Hospital at Westmead, Sydney, Australia; 8) Ottawa Health Research Institute, Ottawa, Ontario, Canada.

Cobalamin C deficiency (*cblC*) is a rare autosomal recessive disorder (OMIM 277400) that usually presents in infancy with failure to thrive, developmental delay, hypotonia, and haematological abnormalities. It is the most common intracellular cobalamin processing disorder. Although vitamin therapies can be considered, diagnosis is difficult due to the diverse symptoms, and their varying degree of severity. DNA from ten families with *cblC* deficiency have been collected with 10 patients and a total of thirty-six individuals. Diagnosis was confirmed through complementation studies. Six affected individuals are offspring of consanguineous marriages predicting that gene mutations are homozygous-by-descent. A genome scan with markers positioned at 25 cM intervals was conducted, along with additional markers to achieve a 10 cM interval scan, for a total of 287 loci. Two-point linkage analyses were performed with each marker yielding positive lod scores for six loci at D1S2134, D1S1609, D2S441, D5S2488, D10S1423 and D16S2616. Five of these were eliminated upon examination of additional neighbouring markers, positioned within 1cM-5cM distances. D1S2134 gave a homogeneity lod score of 2.93 at $q = 0$. Exclusion of all alternate remaining genome regions with multi-point exclusion mapping and the refinement of the interval surrounding D1S2134 is continuing in order to focus our candidate gene analysis and positional cloning effort to identify the gene responsible for *cblC* deficiency.

Feasibility of complex pedigrees in an isolated population for QTL mapping of cholesterol levels. *M. Falchi¹, P. Forabosco¹, E. Petretto¹, A. Angius¹, C. Cappio Borlino², I. Persico¹, G.B. Maestrale¹, M. Fanciulli¹, D. Bebbere¹, D.A. Prodi¹, C. Fraumene¹, M.A. Palmas², P.M. Melis¹, M. Pirastu¹.* 1) Istituto di Genetica delle Popolazioni, CNR, Alghero, Sassari, Italy; 2) SharDNA, Cagliari, Italy.

A powerful approach to mapping complex-disease genes is to study isolated, founder population where genetic and environmental heterogeneity is reduced. Our study was performed on an isolated sub-population from Sardinia, consisting of a unique large, complex pedigree in 16 generations. This population may provide significant power to the identification of QTLs such as serum lipids levels. The study sample included 758 subjects, for whom serum total cholesterol (TC), LDL and HDL cholesterol levels were measured. All individuals were genotyped for a 5 cM microsatellite map (NHLBI Genotyping Service). Standard methods are computationally challenging in extremely large and complex pedigrees and do not account for genetic heterogeneity. We follow a multi-step approach based on increasing pedigree size and complexity, maximizing trait heritability among family members. In the first stage variance components linkage analysis was conducted using five large families (largest of 206 individuals) for a total of 261 phenotyped subjects. Heritabilities were 0.40 for TC, 0.37 for LDL and 0.41 for HDL, all resulting highly significant. The highest multipoint lodscore was found on chromosome 18 ($Z=2.07$) for LDL. The same region was also found to be involved in TC variability ($Z=1.84$). Other regions showing multipoint lodscores >1 were obtained on chromosomes 2, 4 and 18 for TC and LDL, and on chromosomes 1, 3 and 19 for HDL. Among the candidate loci associated with serum lipid levels, linkage evidence was maximized for a large family of 936 individuals on 19p13.2 for TC, near the LDLR locus ($Z=3.24$), thus suggesting a major role for this gene in our population. Our study shows that methods used to simplify the whole pedigree are extremely important. Indeed, even in such a homogeneous isolated population, genetic and environmental heterogeneity among subunits of the pedigree may play a significant role on the power of our studies.

Linkage to chromosome 14q in Alzheimer's Disease (AD) without psychotic symptoms. *D. Avramopoulos¹, M.D. Fallin², S.S. Bassett¹*. 1) Psychiatry, Johns Hopkins University, Baltimore, MD; 2) Epidemiology, Johns Hopkins University, Baltimore, MD.

The genome scans performed so far on Late Onset Alzheimer's Disease (LOAD) have not consistently produced positive findings at any chromosomal locus. The lack of such findings could be due to the genetic heterogeneity of LOAD. One way to reduce heterogeneity is to study additional phenotypic information and use them as covariates in the analysis. A number of new linkage methodologies are now attempting to make use of such covariates. One common finding in AD patients is the presence of psychotic symptomatology, namely hallucinations and delusions. The presence or absence of such symptoms might be related to the genetic background leading to disease and could therefore increase our chance to detect linkage for the disease if it is used as a covariate in the analysis. As part of the NIMH genetics initiative we have collected and performed a 9 cM genome scan on 457 LOAD pedigrees. For the subset of pedigrees collected by Johns Hopkins (n=148), we have information on the presence of such symptoms. We have used the computer program LODPAL, which allows the inclusion of covariates in the non-parametric linkage analysis, taking into account the presence of hallucinations. This analysis provided a strong linkage signal on chromosome 14q around the PS1 locus. The results suggest that the presence of hallucinations has a negative effect on linkage in the region, thus linkage is only present in the absence of hallucinations. SSCP screening of PS1 in 28 individuals linked to the region did not reveal any coding region mutations. Further studies are needed to exclude PS1 and identify the gene linked to LOAD without psychotic symptoms.

Ordered Subset Analysis in Primary Open-Angle Glaucoma (POAG): Evidence for Linkage to Chromosomes 14 and 15. *R.R. Allingham¹, J.L. Wiggs², E.R. Hauser¹, M.A. Hauser¹, F.L. Graham¹, K.R. LaRocque¹, B.W. Broomer¹, E.A. del Bono², J.R. Shi³, J.L. Haines³, M.A. Pericak-Vance¹.* 1) Duke University Medical Center, Durham, NC; 2) Massachusetts Eye and Ear Infirmary, Boston, MA; 3) Vanderbilt University Medical Center, Nashville, TN.

Ordered Subset Analysis (OSA) was used to reduce the impact of phenotypic heterogeneity underlying POAG (Hauser et al. 1998; Ghosh et al. 2000). OSA evaluates evidence for linkage in the presence of heterogeneity. We sought to identify homogeneous family subgroups on the basis of age of diagnosis (AOD). Mean and minimum AOD for the affecteds in a family were analyzed separately as covariates in the OSA analysis.

Eighty-six multiplex families with completed genotype and covariate information were included in this analysis. Multipoint lod scores were calculated using information from all available affected relative pairs via GENEHUNTER-PLUS software package incorporating Kong and Cox's model (Kruglyak et al. 1996; Kong et. al. 1997). These multipoint lod scores were used to calculate the OSA statistic. However, AOD covariate information did yield a significant increase in the LOD scores seen in chromosomes 14 and 15 with empirical p-values ≤ 0.05 . When this data set was analyzed using mean AOD, the peak OSA_LOD score for chromosome 14 was 3.68 (DLOD=1.90) at D14S72 (subset=fourteen families). Similarly, the peak for chromosome 15 was 3.19 (DLOD=1.78) at GABRB3 (subset=sixteen families). We had similar increases in the peak LOD score when we analyzed minimum AOD. There is significant overlap between the families linking to both chromosomes. We have identified homogeneous phenotypic subsets of families that contribute to linkage on both chromosomes 14 and 15. These data provide evidence that AOD is a useful stratification variable in identifying genes in POAG. Support: The Glaucoma Research Foundation, Barkhouser Glaucoma Research Fund, and NIH Grant EY 10886.

POSTERIOR POLAR CATARACT: CLINICAL SPECTRUM AND GENETIC ANALYSIS IN A LARGE FAMILY. *S. Finzi¹, Y.Y. Li¹, T.N. Mitchell¹, A. Farr¹, O. Sundin², I.H. Maumenee¹.* 1) Johns Hopkins Center for Hereditary Eye Disease; 2) Laboratory of Developmental Genetics, Johns Hopkins Medical Institutions, Baltimore, MD.

PURPOSE: Congenital cataracts affect about 6 patients per 10,000 births and frequently cause blindness in infants. Inherited cataracts account for up to half of all congenital cataracts. They are clinically and genetically heterogeneous. Loci for autosomal dominant posterior polar cataracts have been mapped to chromosomes 1p36, 16q22, 20p12-q12 and 11q22-q22.3. The purpose of this study is to present the clinical findings and the genetic analysis in a large family with autosomal dominant congenital posterior polar cataracts. **METHODS:** A four-generation American family with congenital posterior polar cataracts was identified. Forty-four members of the family were examined ophthalmologically and a total of 20 patients were diagnosed with congenital cataracts. Blood samples were collected with consent for genetic analysis of 28 patients of whom 15 were affected. After exclusion of known loci for posterior polar cataract, a genome-wide screen was conducted with a set of 170 markers spaced at 25-cM intervals. **RESULTS:** We mapped dominant congenital posterior polar cataract to chromosome 10q24. The maximal LOD score of 4.52 at recombination fraction $q = 0$ was obtained for marker D10S574. On haplotype analysis a 13cM interval between loci D10S1680 and D10S467 was identified, which included the PITX3 gene. On sequencing the coding region of PITX3, we found a 17 base pair duplication in exon 3. The mutation was co-inherited with the disease in all instances. The insertion resulted in a frameshift, altering 82 C-terminal amino acids. **CONCLUSION:** This study provides further evidence for genetic heterogeneity of autosomal dominant posterior polar cataract. Although Semina EV(1998) described the same genotype in one patient with anterior segment mesenchymal dysgenesis (ASMD) with cataracts, the common phenotype of this mutation is probably posterior polar cataract and a modifier gene could cause the anterior segment abnormalities in the previously described patient.

Evidence for interaction between CARD15 and the IBD4 locus (chr. 14) in Crohns disease families. *Y. Lin, K.D. Taylor, T. Hang, N. Fischel-Ghodsian, J.I. Rotter, H. Yang.* Medical Genetics, Birth Defects Ctr, Cedars-Sinai Medical Ctr, Los Angeles, CA, USA.

Background: Genome-wide searches for inflammatory bowel disease (IBD) susceptibility genes by multiple centers have identified putative Crohn's disease (CD) loci on chr. 6 (IBD3), 12 (IBD2), 14 (IBD4) and 16 (IBD1). CARD15 was recently identified to be the CD IBD1 gene. The aim of this study was to examine the potential interaction between CARD15 and other putative CD loci. **Methods:** 91 Caucasian CD multiplex sibpair families (sibship size 2: n=78, 3:12, 4:1) were genotyped for 3 CD associated CARD15 SNPs (R675W, G881R, and 980fs) and 18 microsatellite markers spanning the IBD2, IBD3 and IBD4 loci with a mean spacing of 7 cM. Two-point linkage analyses using SIBPAL in SAGE were performed in all families and by stratifying on CARD15 genotype. Families with at least one individual with a CARD15 mutation were CARD15+ (n=50; CARD15- n=41). Independent sample T-test was used to compare mean allele sharing (MAS) at each marker between groups. **Results:** In all CD families, borderline to significant linkage was observed across 8 markers at IBD2 (MAS=0.54-0.62, p=0.09-0.00002). Significant linkage was observed at IBD3 at D6S289 and D6S1060 (MAS=0.55, 0.56; p=0.03-0.04). MAS at IBD4 ranged 0.49-0.53 (p=0.08 at D14S261). For IBD3, CARD15- appeared to have a higher mean MAS (0.51-0.58) than CARD15+ families (0.48-0.55), although the differences were not statistically significant. Most interesting, for IBD4, the CARD15+ MAS (0.52-0.58) was consistently higher than CARD15- (0.44-0.52) at all 4 markers. In particular, MAS at D14S261 showed a significant difference between CARD15+ and CARD15- families (0.58 vs. 0.48, p=0.047) indicating a potential interaction between CARD15 and the IBD4 locus. **Conclusions:** There appears to be an interaction between CARD15 and the IBD4 locus (on chr. 14). CD families with a CARD15 mutation have a higher mean allele sharing for the IBD4 locus than those without. This study demonstrates that stratifying on CARD15 genotype may help to dissect the genetic factors predisposing to CD and thereby help identify other genes responsible for the disease.

An initial genome scan for type 1 Diabetic Nephropathy susceptibility loci in the Finnish population. *A.M. Osterholm¹, B. He¹, J. Pitkäniemi², I. Reynisdóttir³, T. Berg¹, J. Tuomilehto², K. Tryggvason¹.* 1) MBB, Matrix Biology, Karolinska Institute, Stockholm, Sweden; 2) The Finnish National Public Health Institute, Helsinki, Finland; 3) DeCODE Genetics, Reykjavik, Iceland.

Diabetic nephropathy (DN) is one of the severe complications affecting patients with type 1 and type 2 diabetes, and is the main cause of end stage renal disease in the Western world. DN is pathologically characterized by thickening of the glomerular basement membrane (GBM), which distorted the glomerular filtration barrier. Clinically, it is manifested by persistent proteinuria and progresses to impaired renal functions. However, the pathogenic mechanisms of DN are still unknown. Several epidemiological studies have provided evidence for genetic determinants of nephropathy. Loci linked to DN have been suggested on chromosomes 3, 7, 9 and 20, but no specific genes have been reported to be directly associated with DN. In the present study, our aim is to search for genes responsible for type 1 diabetic nephropathy using a genome-wide scan strategy. Sib pair families were collected from Finland. A dense marker map consisting of 900 markers with an average spacing of 4 cM was used. Discordant sib pairs (DSP), where siblings are concordant for type 1 diabetes, but discordant for nephropathy, were applied to map disease loci. In an initial genome wide scan, 868 markers, covering the 22 autosomal chromosomes plus the X chromosome, were successfully genotyped in 71 Finnish DSPs. Linkage analysis suggested four loci with a maximum lod score (MLS) above 1 on four chromosomes (3q, 4q, 16q and 22p). The highest observed MLS was 2.3 ($p=5.7 \times 10^{-4}$) on 4q. The locus on 3q (MLS 1.7) is a confirmation of a previous finding in an American Caucasian population. This locus has been investigated further in a separate study. Another two loci on 16q and 22q showed MLS of 1.44 and 1.45 respectively. Follow-up studies with additional markers as well as families will be carried out on chromosomes 4, 16 and 22. These three loci have not been reported previously and the suggested linkage will be investigated.

No evidence for a locus influencing Lp(a) levels on chromosome 1. *B.T. Heijmans¹, M. Beekman¹, N.G. Martin², J.B. Whitfield³, N.L. Pedersen⁴, U. DeFaire⁴, H. Snieder⁵, N. Lakenberg¹, P. de Knijff⁶, R.R. Frants⁶, G.J.B. van Ommen⁶, C. Kluit⁷, G.P. Vogler⁸, P.E. Slagboom¹, D.I. Boomsma⁹.* 1) Molecular Epidemiology, Leiden University Medical Centre, Leiden, The Netherlands; 2) Queensland Institute for Medical Research, Brisbane, Australia; 3) Clinical Biochemistry, Royal Prince Alfred Hospital, Sydney, Australia; 4) Medical Epidemiology, Karolinska Institute, Stockholm, Sweden; 5) Georgia Prevention Institute, Medical College of Georgia, Augusta, United States of America; 6) Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands; 7) TNO Prevention and Health, Leiden, The Netherlands; 8) Biobehavioral Health, Center for Developmental and Health Genetics, Pennsylvania State University, PA, USA; 9) Biological Psychology, Free University of Amsterdam, Amsterdam, The Netherlands.

Plasma levels of lipoprotein(a) (Lp(a)) were long believed to be influenced by the LPA locus at chromosome 6q27 only. A recent report, however, suggested the presence of a second locus on chromosome 1 influencing Lp(a) (Broeckel et al. Nat Genet 2002;30:210). We performed a linkage study aimed at replication of this finding in 1445 twin pairs, of whom 483 were dizygotic and 962 monozygotic. As expected, we obtained highly significant evidence for linkage (LOD=9.8) with Lp(a) plasma levels at the LPA locus on chromosome 6. Next, we analysed chromosome 1 and a LOD score of 1.6 was obtained at 251 cM from pter. However, the two putative QTLs at chromosome 6 and 1 together explained 126% of the variation in Lp(a) levels (44% and 82% for chromosome 1 and 6, respectively), which likely resulted from an overestimation of effect sizes, a common phenomenon in genome-wide linkage analyses. We, therefore, modelled both putative QTLs simultaneously. When we analysed this two-locus model, the positive LOD score at chromosome 1 completely disappeared. Moreover, the chromosome 1 locus now explained none of the variation in Lp(a) plasma levels. Our data provide no evidence for an additional locus at chromosome 1 influencing Lp(a) plasma levels and we are thus unable to replicate the previously reported linkage result.

A second generation genome scan for rheumatoid arthritis. *J. Osorio y Fortea*¹, *C. Pierlot*¹, *E. Petit-Teixeira*¹, *S. Cailleau-Moindrau*¹, *C. Sardou*¹, *C. Stalens*¹, *P. Dieudé*¹, *G. Bana*¹, *T. Bardin*², *F. Clerget-Darpoux*³, *B. Prum*⁴, *F.B. Cornélis*^{1,2}. 1) GenHotel / Laboratoire de Recherche pour la Polyarthrite Rhumatoïde, 91057, Evry, Université Paris7 - université d'Evry, France; 2) Unité de Génétique Clinique, Hôpital Lariboisire, Assistance Publique Hôpitaux de Paris, Université d'Evry, France; 3) INSERM, Hôpital Kremlin Bicêtre 78-80 rue du Général Leclerc, 94276, Le Kremlin Bicêtre, France; 4) Laboratoire Statistique et Génome, Génopôle, tour Evry2 , 91000, Evry, France.

Background : Rheumatoid Arthritis (RA, OMIM number180300) is the most frequent autoimmune disorder (1% prevalence). Searching for susceptibility loci outside the HLA region, genome scans have been reported in French, Japanese, American and English RA families. The genetic information extracted was only partial, and the resolution was from 10 to 12 cM. As these scans used different genetic map, a meta-analysis is difficult. **Objective :** To refine our genome scan to a resolution below 4 cM, in order to extract all genetic information and facilitate future meta-analysis. **Methods :** 88 Caucasian Affected Sib Pair (ASP) families were genotyped with 1109 microsatellite markers (77% mean heterozygosity) defining the finest panel ever used in a genome scan for a human disease (3.5 cM). Fluorescent technology was used on ABI sequencer with GenScan and Genotyper softwares. Multipoint model free linkage analysis was performed using GeneHunterPlus program. **Results :** Preliminary analysis of the first 1000 markers showed linkage in the HLA region (lowest p-value = 0.0007) and suggested loci (p-value < 5%) different than those observed in our initial 12 cM scan. Altogether, 66 markers defining 12 non-overlapping loci were observed, resulting in 150 Mb of genome sequence, *i.e.* about 1500 potential candidate genes. **Conclusion :** We have refined our RA genome scan to a resolution below 4 cM, allowing us to extract all genetic information and facilitate future meta-analysis.

No linkage in the FSH-receptor region for age at natural menopause in Dutch sibpairs. *H.S. Kok^{1,2,4}, K.M van Asselt^{1,2,4}, L.A Sandkuijl³, P.H.M Peeters², Y.T van der Schouw², E.R te Velde⁴, D.E Grobbee², P.L Pearson¹.* 1) Department of Medical Genetics, University Medical Center, Utrecht, The Netherlands; 2) Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands; 3) Department of Medical Statistics, Leiden University Medical Center, Leiden, The Netherlands; 4) Department of Reproductive Medicine, University Medical Center Utrecht, Utrecht, The Netherlands.

Objectives: Age at natural menopause is a retrospective marker for timing of reproductive events such as onset of sub- and infertility. Natural age at menopause exhibits a 20-year spread in the population. Several studies have noted previously that environmental factors account for only a small part of the variation in age at menopause. Heritability estimates of 0.53 and 0.63 have been reported for age at menopause in twin studies and an estimate of 0.85 for sisters. Our aim is to locate regions in the genome that might contain genes contributing to the genetic variation in age at menopause by performing a whole genome scan. However, in women exhibiting premature ovarian failure (age at menopause before age 40), a study has previously reported association with the FSH-receptor. This could be a candidate gene for the normal variation in age at menopause. Preceding the genome scan, we performed a sibpair study with 5 markers in the FSH-receptor region. Materials and methods: Blood samples of pairs of menopausal sisters with ages at menopause in the extremes of the natural menopause age distribution (before 45 or after 55 years) were collected. Where possible, parents or additional siblings were included for phase-determination. DNA was isolated and information on factors that could influence age at menopause was collected from all siblings. The Haseman-Elston as well as a variance components model were applied to analyze the data. Results: The total number of included families is 124. We detected no linkage in the FSH-receptor region for age at menopause.

A genome scan for allergic asthma and related phenotypes in an Italian population sample. *G. Malerba¹, E. Trabetti¹, C. Patuzzo¹, C. Migliaccio¹, R. Galavotti¹, M.C. Lauciello¹, L. Xumerle¹, L. Pescollderungg², A.L. Boner¹, M. Lathrop³, P.F. Pignatti¹.* 1) Mother-Child & Biol, Genetics, Univ Verona, Verona, Italy; 2) Institute of Paediatrics, Hospital of Bolzano, Bolzano, Italy; 3) Centre National de Genotypage, Paris, France.

A genome scan with 394 microsatellite polymorphic markers has been performed on 123 Italian families (604 individuals genotyped) ascertained through an allergic asthmatic child. Four qualitative phenotypes related to allergy or asthma have been investigated: asthma, bronchial hyperresponsiveness to methacholine challenge (BHR), total serum elevated IgE and skin prick test positivity to common allergens (SPT). Statistical analysis was performed by non parametric linkage analysis implemented in the computer program Merlin. Markers on 7 sites of chromosomes 1, 4, 9, 11, 13 and 15 showed a suggestive linkage ($p < 0.01$) with at least one of the 4 phenotypes investigated: D1S213 with elevated IgE ($p = 0.0012$), D4S426 with asthma ($p = 0.003$), D9S286 with elevated IgE ($p = 0.0006$) and SPT ($p = 0.001$), D9S1690 with elevated IgE ($p = 0.004$), D11S4191 with BHR ($p = 0.008$), D13S156 with elevated IgE ($p = 0.0004$) and D15S127 with asthma ($p = 0.009$). These preliminary results will be further investigated looking for two loci interactions and performing transmission disequilibrium tests using markers used for this genome scan, and additional biallelic markers located in the linked regions.

Genetic linkage analysis of ADPKD1 and ADPKD2 in several Iranian families using highly polymorphic microsatellite markers. *A. Hajibeigi¹, R. Radpour¹, M.M. Haghghi¹, M. Ohadi¹, B. Broumand², A.R. Afzal³, H. Najmabadi¹.* 1) Genetic Research Center, U. of Social Welfare and Rehab, tehran, tehran, Iran; 2) Rasoul Akram U.Hospital. Medical Science of Iran university. Tehran. Iran; 3) Medical Genetics Unit, St George's Hospital Medical School, London, UK.

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common genetically heterogeneous disorders affecting human with the incidence of about 1:1000 in all population. Three loci have been identified causing disease. One located on the short arm of chromosome 16 (ADPKD1) accounts for about 85-90% of the PKD patients, and ADPKD2 gene on chromosome 4 that approximately accounts for 15% of the cases. ADPKD3 has been identified but with unknown genomic locus. In this study the linkage analysis was performed using KG8, 16AC2.5, SM7, D4S1534, D4S231 and D4S423 markers linked to both ADPKD1 and ADPKD2 in five affected Iranian families. In parallel to this study, the genomic DNA of 50 unrelated healthy individuals were used to determine frequency, heterozygosities and PIC for each marker. Our results showed the highest heterozygosities and PIC values for all markers and the most informative markers were KG8 and SM7 for PKD1 and D4S231 for PKD2. Our data indicate that three of the five families had linkage to PKD1 whereas one family was linked to PKD2 gene. The other one was uninformative due to the family structure.

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A high throughput and accurate method for SNP genotyping using Sequenom MassARRAY™ system. *K. McKernan, C. Fujii, J. Ziauddin, J. Malek, P. McEwan.* Agencourt Bioscience Corp., Boston, MA.

Mass spectrometry has proven to be a rapid, easily multiplexed, and highly accurate SNP genotyping technology. An obstacle of mass spectrometry-based technologies can be the expense of the required instrumentation. Agencourt Biosciences SeeSNP™ Genotyping method is a multi-stage process based on the Sequenom MassARRAY platform, which combines primer extension technology with MALDI-TOF mass spectrometry. By measuring the molecular weight of primer extension products using mass spectrometry, the MassARRAY system is capable of SNP genotyping with unprecedented levels of accuracy without the need for labeling or separation steps. The MassARRAY platform combined with Agencourts genomic expertise and fully-automated facility create a cost effective SNP genotyping service which provides the high quality, high throughput SNP analysis necessary for the completion of large scale pharmacogenomic studies.

SNP Analysis of Candidate Genes Associated with Type 2 Diabetes in Chinese Han Population. *H. Sun¹, J. Zhao², W. Du¹, H. Wang³, J. Zuo¹, B. Qiang⁴, Y. Shen⁴, Z. Yao⁴, W. Huang⁵, Z. Chen⁵, M. Xiong², F. Fang¹.* 1) Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100005, China; 2) Human Genetics Center, The University of Texas, Health Science Center, Houston TX 77225, USA; 3) Peking Union Medical College Hospital, Beijing 100730, China; 4) Chinese National Human Genome Center at Beijing, Beijing 100176, China; 5) Chinese National Human Genome Center at Shanghai, Shanghai 201203, China.

In order to clone the susceptibility genes of type 2 diabetes in Chinese Han population in the 1p36.33-36.23 region that localized by genome screening in our former research, we conducted case-control study by using the third generation of genetic marker, single nucleotide polymorphism (SNP). The results showed that 3 SNPs, resided in PRKCZ, UTS2 and SLC2A5 gene respectively, might be associated with the disease. There was statistical difference of their allele frequency between case and control groups, especially the SNP rs436045 in PRKCZ gene, which showed significant discrepancy and implied possible association with the disease. A set of SNPs located upstream and downstream from rs436045 in PRKCZ gene were selected to conduct case-control study and linkage disequilibrium (LD) analysis. The results suggested that 5 SNPs extending about 7kb were in the same haplotype block and there was significant discrepancy of their haplotype frequency between case and control groups. To search for the causal loci, we constructed 10 reporter plasmids containing different alleles of the 5 SNPs and transferred them to HepG2 cell. As a result, the reporter gene activity of different alleles of 2 SNPs, rs427811 and rs809912, were obviously different. We infer that these 2 SNPs might be susceptibility loci of the disease. They play a role in the pathogenesis of the type 2 diabetes by affecting the expression level of PRKCZ gene.

Chromosomal mapping of Essential Tremor. A. Shatunov¹, J. Jankovic², R. Elble³, N. Sambuughin⁴, Y. Zhang⁵, A. Dagvadorj¹, J. Ji⁵, H-S. Lee¹, M. Hallett¹, L.G. Goldfarb¹. 1) National Institute of Neurological Disorders and Stroke, Bethesda, MD; 2) Baylor College of Medicine, Houston, TX; 3) Southern Illinois University School of Medicine, Springfield, IL; 4) Barrow Neurological Institute, Phoenix, AZ; 5) BBI Biotech Research Laboratories, Gaithersburg, MD.

Hereditary essential tremor (ET) is the most prevalent movement disorder that occurs in about 1 of 200 to 300 people. ET is a heterogeneous entity expressed by phenotypically distinct types of disease: monosymptomatic postural or action tremor that increases during active movement and disappears at rest (T), and tremor combined with variably expressed focal dystonia (T-D). Both types are inherited with an autosomal dominant pattern. The T-type of ET was recently mapped to loci at 3q13 and 2p25, but analysis of other reported families did not support linkage to these locations. To further genetic characterization of ET we performed genome-wide scan in two large American families. Family A included 12 definitely affected individuals, most of them with the T-D phenotype. Seven patients in family B had T phenotype. Definite ET patients were considered as affected and all other family members were designated as unknown. GENEHUNTER and a newer MERLIN packages were used for multipoint calculations with both non-parametric (NPL) and parametric (LOD) statistics and exact compound P-values. Chromosome maps were adopted from the Marshfield web site with some corrections based on NCBI sequences in the candidate regions of the genome. Family A showed suggestive linkage at locus 6p22 (GENEHUNTER: NPL=2.70, p=0.003; LOD=1.78; MERLIN: NPL=2.89, p=0.002; LOD=1.21, p=0.009). The highest scores in family B were observed at two loci: 3p14 (GENEHUNTER: NPL=4.61, p=0.015; LOD=1.88; MERLIN: NPL=3.14, p<0.001; LOD=0.76, p=0.03) and 6p24 (GENEHUNTER: NPL=4.61, p=0.015; LOD=1.88; MERLIN: NPL=4.71, p<0.001; LOD=0.91, p=0.02). The distance between the markers showing maximal scores in families A and B within the 6p22-p24 locus is approximately 18.5 cM.

A genome-wide scan in type 2 diabetes mellitus provides independent replication of a susceptibility locus on 18p11 and suggests existence of novel loci on 2q12 and 19q13. C. Wijmenga¹, J.H. van Tilburg¹, L.A. Sandkuij^{1, 3}, E. Strengman¹, P.L. Pearson¹, T.W. van Haefen². 1) Dept Medical Genetics, Univ Medical Ctr, Utrecht, Netherlands; 2) Dept Internal Medicine, Univ Medical Ctr, Utrecht, Netherlands; 3) Departments of Medical Statistics, Leiden Univ Medical Ctr, Leiden, Netherlands.

It is well established that diabetes mellitus is a multifactorial disease, in which many genes interact not only with each other but also with environmental factors. Factors such as age, physical activity, diet, and obesity play a major role in the disease aetiology. We performed a genome-wide scan in Dutch Caucasian sibpairs with type 2 diabetes (T2D), with the aim of identifying susceptibility loci in the Dutch population. We found an interesting LOD on chromosome 18, in a region previously described by Parker et al. in a BMI-stratified subset of Finnish/Swedish patients. When we made a similar stratification our LOD increased from 0.7 to 2.3 between D18S471 and D18S843 in the 20% most obese pedigrees (DS-20%). This finding indicates that our data set consists of two subtypes, an obese pedigree group and a lean group. Analysis of the 80% lean pedigrees (DS-80%). revealed modest indications of linkage to regions on chromosomes regions 2q12 and 19q13 (multipoint LOD scores of 1.5 and 1.2, respectively). Although these loci do not reach suggestive LOD scores, they might indicate the existence of novel loci involved in T2D in the Dutch population. Our results for chromosome 18 clearly replicate the linkage to this region found in a Finnish/Swedish population and previously reported by Parker et al. Combined with our finding it indicates that a novel gene resides in the 18p11 region, which forms part of an, as yet unidentified, pathway involved in T2D and obesity.

Genome-wide scan in a subset of obese type 2 diabetes patients and subsequent QTL mapping for BMI suggests linkage to two distinct loci on chromosome 11. *J.H.O. van Tilburg¹, L.A. Sandkuijl^{1,3}, E. Strengman¹, P.L. Pearson¹, T.W. van Haeften², C. Wijmenga¹.* 1) Dept Medical Genetics, Univ Medical Center, Utrecht, the Netherlands; 2) Dept Internal Medicine, Univ Medical Center, Utrecht, the Netherlands; 3) Dept Medical Statistics, Leiden Univ Medical Center, Leiden, the Netherlands.

It is well established that type 2 diabetes mellitus (T2D) is a multifactorial disease, in which many genes interact not only with each other but also with environmental factors. Factors such as age, physical activity, diet, and obesity play a major role in the disease etiology. Like diabetes, obesity is also a complex trait that is determined by multiple genetic and environmental factors. In recent years, several single-gene defects responsible for obesity in rodents and, in rare cases, for human obesity have been identified. To further study diabetes and obesity, we undertook a genome-wide analysis for diabetes in the most 20% obese families from a Dutch Cohort of T2D patients and stratified the whole data set by BMI. We looked for susceptibility loci involved in diabetes and subsequently searched for quantitative trait loci (QTL) influencing obesity using variance components (VC) analysis. The most promising regions found so far in both the genome-wide scan and in the QTL mapping for BMI are on chromosome 11. A maximum LOD score of 1.7 was obtained between markers D11S2362 and ATA34E08 (region 11p15-13) in the genome-wide scan and a VC LOD score of 2.4 was obtained between markers D11S940 and D11S2000 (region 11q22) in the QTL mapping. Although the loci are 100 cM apart, both are interesting. It was shown by Hani et al. that the region 11p15-13 harbors the SUR1 gene, which is a key component in glucose-stimulated insulin secretion and also showed that a certain variant of SUR1 is associated with morbid obesity and T2D. On region 11q22 linkage was reported with BMI and T2D in Pima Indians. We therefore conclude that the two loci on chromosome 11 are involved in both obesity and T2D; further research is necessary in this region to elucidate the implications of the loci.

Linkage of Autistic Disorder to chromosome 15q11-q13 using phenotypic subtypes. *Y. Shao¹, M.L. Cuccaro¹, E.R. Hauser¹, K.L. Raiford¹, C.M. Wolpert¹, S.L. Donnelly¹, L.A. Elston¹, S.A. Ravan², R.K. Abramson², H.H. Wright², G.R. DeLong¹, J.R. Gilbert¹, M.A. Pericak-Vance¹.* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC 27710; 2) W.S. Hall Psychiatric Institute, University of South Carolina, Columbia, SC 29202.

Autistic disorder (AutD) is a complex genetic disease. It is postulated that several genes contribute to the underlying genetic risk of developing AutD. However, both etiologic and genetic heterogeneity confound the discovery of these AutD susceptibility genes. The chromosome 15q11-q13 has been identified as a candidate region based on both the frequent occurrence of chromosomal abnormalities and suggestive linkage and association findings. Ordered subsets analysis (OSA) is a novel method to identify a homogeneous subset of families that contribute to linkage in a chromosomal location. Thus OSA can be potentially used to help fine map and localize the susceptibility gene in a chromosomal area. A component representing insistence on sameness (IS), derived from a principle component analysis using data of 221 AutD patients from the repetitive behaviors/stereotyped patterns domain in the ADI-R, was used as a covariate in OSA analysis. Families sharing severe degree of IS (~25% of the 81 total AutD multiplex families) increased the linkage evidence on 15q11-q13 at the GABRB3 locus from LOD score 1.45 to 4.71. These results support previous reports implicating the involvement of g-Aminobutyric Acid Receptor Subunit genes in AutD and further show that the homogeneous subtype in AutD increases the power to map disease susceptibility genes in complex traits.

The Third Genetic Locus (GLC3C) for Primary Congenital Glaucoma (PCG) Maps to Chromosome 14q24.3. *M. Sarfarazi, I. Stoilov.* Molecular Ophthalmic Genetics Laboratory, Surgical Research Center, University of Connecticut Health Center, Farmington, CT.

A five-generation consanguineous family segregating for Primary Congenital Glaucoma (PCG) in an autosomal-recessive manner was ascertained and samples were obtained from 13 available individuals. Family members were genotyped with a group of polymorphic DNA markers flanking the two previously published PCG loci of *GLC3A/CYP1B1* on 2p21 and *GLC3B* on 1p36. Critical recombination events indicated that this PCG family is not linked to either of these two loci. In order to identify the chromosomal location for this family, we next initiated a genome-wide scan. Automated fluorescent genotyping was performed with the CHLC/Weber human screening set (Version 9; Research Genetics). Genotyping of 235 polymorphic markers identified only one (D14S53) that showed reduction in homozygosity in all PCG affected individuals as compared to the unaffected members. Saturation mapping and haplotype analysis with 6 additional markers (D14S61, D14S42, D14S983, D14S1020, D14S74 and D14S1000) revealed that all affected individuals share a region of homozygosity that is defined by markers D14S42, D14S983, D14S1020 and D14S74. Statistical evidence for linkage evaluation was carried out with MLINK subcomponent of the LINKAGE package (FASTLINK version). The highest two-point LOD score of 2.44 was obtained with a CA-repeat marker (ERR2b) that is located at the 3-prime end of Estrogen-Related Receptor Beta (*ESRRB*) gene. Four-point linkage analysis between PCG and DNA markers of ERR2b, ERR2a (a CA-repeat marker at the 5-prime end of *ESRRB* gene) and D14S74 produced a maximum LOD score of 3.09. Therefore, our data suggests that the third PCG locus (GLC3C) is located on chromosome 14q24.3, within a region of approximately 2.9 cM that is flanked by D14S61 and D14S1000. The physical distance between these two markers is estimated to be around 6 Megabases. There are more than 20 known genes within the newly identified GLC3C interval including, Neurexin-3 (*NRXN3*) and *ESRRB*. Mutation screening of these two candidate genes is currently in progress. Supported by a NIH grant (EY-11095) to MS.

The Power of Genome-Wide Haplotype Analyses for Complex Traits. *B.K. Rana*¹, *S.B. Gabriel*², *S.F. Schaffer*², *M.J. Daly*², *D. Altshuler*^{2,3}, *N.J. Schork*¹. 1) Polymorphism Research Laboratory, Dept of Psychaitry, Univ California, San Diego, La Jolla, CA; 2) Whitehead MIT Center for Genome Research, Cambridge, MA; 3) Departments of Genetics and Medicine, Harvard Medical School; Department of Molecular Biology and Diabetes Unit, Massachusetts General Hospital, Boston, MA.

The haplotype map effort aims to facilitate the discovery of disease-predisposing genes via association mapping. Association mapping, however, is complicated by a number of factors, not the least of which is the fact that, in the absence of a prior knowledge about the location of a disease gene, one may have to test literally tens of thousands of possible genomic sites for association with the disease. Multiple testing of this sort can potentially lead to many false positive results. Although the haplotype map project will yield information that will allow us to eliminate such redundant tests, the power of this approach has not been adequately addressed. Here we use the analytic strategy recently outlined by Schork (*American Journal of Human Genetics* (2002) 70:1480-1489) and the haplotype block data recently described by Gabriel et al. (*Science* (2002) May 23) to assess the power of genome-wide association studies that make use of haplotype information. Our results describe the likely performance of and thus requirements for the discovery of disease genes by haplotype mapping approaches.

Linkage analysis of 3 candidate gene polymorphisms with bone density in a sample of Italian families. A.

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Osteoporosis is one of the major and growing health care problems around the world largely related to the general aging of developed countries populations. Several studies have reported that 60-80% of the variance in bone phenotype measurement is genetically determined. Several case-control studies in candidate genes reported association of vitamin D receptor (VDR), collagen 1 alpha1 (COL1A1) and estrogen receptor (ER) gene polymorphisms with bone phenotype. A sample of 119 Italian families (563 individuals genotyped) ascertained through an osteoporotic individual has been genotyped for the ER-PvuII, VDR-FokI, and COL1A1-Sp1 polymorphisms. A variance component linkage analysis has been performed for bone density z-score values of femoral neck, spine, and total hip. The preliminary results indicate that none of the polymorphisms investigated is significantly linked to bone density levels of the 3 sites studied: COL1A1-SpI: $p=0.07$, $p=0.08$, $p=0.5$ at spine, femoral neck and total hip, respectively; VDR-FokI: $p=0.5$, $p=0.4$, $p=0.4$ at spine, femoral neck and total hip, respectively; ER-PvuII: $p=0.3$, $p=0.14$, $p=0.07$ at spine, femoral neck and total hip, respectively. Additional genetic markers in the genes, a transmission disequilibrium test for quantitative traits and a more sophisticated analysis taking into account possible covariates could help in dissecting the association of the genes with bone phenotypes in this sample of Italian families.

A Genome Scan For Lupus-Related Autoimmunity Genes. *P.S. Ramos*¹, *J.A. Kelly*², *C.M. Meyer*¹, *A.N. Leiran*¹, *W.A. Ortmann*¹, *J.B. Harley*^{2,3,4}, *K.L. Moser*¹. 1) Department of Medicine, Division of Rheumatic and Autoimmune Diseases, University of Minnesota, Minneapolis, MN; 2) Arthritis and Immunology Program, Oklahoma Medical Research Foundation, Oklahoma City, OK; 3) Department of Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, OK; 4) US Department of Veterans Affairs Medical Center, Oklahoma City, OK.

The possibility that different autoimmune phenotypes may share underlying genetic components has been suggested by several studies. Pedigrees containing systemic lupus erythematosus (SLE) probands are known to have an increased incidence of other autoimmune diseases (~13%), as well as autoimmune serologic abnormalities relative to normal controls. The goal of this study was to identify loci that predispose to humoral autoimmunity in human SLE. Based on the presence of specific autoantibodies commonly associated with SLE, we have defined a novel phenotype for lupus-related autoimmunity (LRA). From our collection of 229 pedigrees multiplex for SLE, we reclassified 1668 total subjects (525 SLE affecteds) for the LRA phenotype and genotyped 279 markers. A total of 850 individuals were reclassified as having the LRA phenotype. We used the revised Haseman-Elston algorithm (SIBPAL) to identify regions of increased allele sharing within these pedigrees. In the European-American families the strongest evidence for linkage was found at 7p15 (D7S1808, $p=0.0005$), 7q21 (D7S2204, $p=0.0005$) and 15q25 (D15S211, $p=0.0016$). The strongest evidence for linkage in the African-American pedigrees was found at 12q24 (D12S2070, $p=0.0006$). Linkage to 7p15 ($p=0.002$), 12q24 ($p=0.008$) and 15q25 ($p=0.0048$) was also observed when all the pedigrees were analyzed together. Comparison of our data to previously reported results indicates some overlap with linkages previously identified in SLE (7q21), rheumatoid arthritis (7p15) and inflammatory bowel disease (12q24), while the effect at 15q25 appears to be novel. These results provide evidence of the presence and locations of genes that are involved in the susceptibility to development of an intermediate autoimmune phenotype and are likely to help further unravel the complexity of SLE as well as other autoimmune diseases.

A genome-wide scan for age-related maculopathy shows evidence of linkage on chromosome 12. *J.H. Schick¹, S.K. Iyengar¹, K. Reading¹, R. Liptak¹, C. Millard¹, K. Lee², S. Tomany², R. Klein², R.C. Elston¹, B.E. Klein².* 1) Epidemiology & Biostatistics, Case Western Reserve Univ, Cleveland, Ohio; 2) Department of Ophthalmology and Visual Sciences, University of Wisconsin Medical School, Madison, Wisconsin.

Age-related maculopathy (ARM), a multifactorial disorder characterized by loss of peripheral central vision, is a leading cause of blindness in the elderly. The importance of genetics in its etiology has been demonstrated by family and twin studies. We conducted the first stage of a genome-wide linkage scan in a selected sample by genotyping 353 autosomal markers in 325 participants comprising 257 sib pairs in the Beaver Dam Linkage Studies of Age-Related Ocular Disorders, including six markers from two candidate gene regions identified on chromosome 1, plus 23 second stage fine mapping markers on chromosome 12. Multipoint linkage analysis was performed using SIBPAL (S.A.G.E. 4.1) to identify regions linked to this degenerative disease. We took as the dependent variable a modified Wisconsin severity scale which is dependent on the size, type, and area of retinal drusen, pigmentary abnormalities, geographic atrophy and exudative macular degeneration. We adjusted this quantitative trait for age and age² prior to conducting the linkage analysis. There was empirical evidence of linkage on chromosome 12: at D12S1300 ($p = 0.0304$), D12S309 ($p = 0.0500$), D12S1671 ($p = 0.0241$), D12S346 ($p = 0.0034$), D12S1588 ($p = 0.0154$), D12S727 ($p = 0.0241$), PAH ($p = 0.0177$), D12S360 ($p = 0.0180$) and D12S338 ($p = 0.0351$). In addition, seven markers located on three other chromosomes were found to be significant for linkage with ARM at a level of $p = 0.05$: chromosome 3 at D3S1259 ($p = 0.0455$), D3S1744 ($p = 0.0209$), and D3S1763 ($p = 0.0004$); chromosome 5 at D5S2500 ($p = 0.0261$); chromosome; and chromosome 16 at D16S769 ($p = 0.0045$), D16S539 ($p = 0.0160$) and D16S621 ($p = 0.0208$). Second stage fine mapping is also being conducted in regions of chromosomes 3, 5 and 16 that demonstrate possible evidence of linkage. This study is supported in part by U.S.P.H.S. research grants GM28356 and EY10605, resource grant RR03655 and training grant HL07567.

Characterization of Asthma, Atopy and Associated Phenotypes in Multiplex Asthma Families. *M.C. Speer¹, P.J. Helms², W. Anderson³, K. Barnes², A. Berg², S. Brewster³, K. Carlsen², J. Gerritsen², C. Haynes¹, W. Lenney², P. Manasco³, A. Roses³, M. Pericak-Vance¹, M. Silverman², P. Sly², J. Sundry², J. Tsanakas², J. Vance¹, M. Hauser¹, M. Whyte².* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Genetics of Asthma International Network; 3) Glaxo-Smith-Kline, Research Triangle Park, NC, and Greenford, UK.

Asthma is a common, complex disorder with genetic and environmental components. We have established an international network of 11 sites, each collecting 100 nuclear families with at least two siblings (age 7-35) with a physician diagnosis of asthma (PDA). We have begun to characterize the genetic contributions to asthma first via genomic screening. In all subjects (including parents) the following measurements were performed: respiratory questionnaire including detailed family history and environmental risk factor assessment; bronchial reactivity (PC20-methacholine); reversibility of airway obstruction; skin-prick tests and serum total IgE. Genomic screening of 365 families of Caucasian descent was performed with 389 markers, an approximate 7 cM genomic screen. Eight phenotypes were considered including PDA, atopic asthma, atopy-broad (at least one SPT ³ 3 or IgE > 100 IU), atopy-conservative (at least one SPT ³ 3), strict asthma (2 of 3 symptoms + BHR), BHR (bronchial hyperresponsiveness), log IgE, and slope of the methacholine dose-response curve. We identified 11 regions of interest in which at least one phenotype generated a lod score ³ 2.0. The most interesting regions include those spanned by D8S1136-D8S1132 (max lod of 2.5 with positive results for 5 phenotypes), 21pter-21qter (max lod score of 2.5 with positive results for 3 phenotypes), and D15S816-D15S87 (max lod score 2.2 with positive lod scores for 3 phenotypes). The highest lod score of 2.8 was obtained for IgE, spanned by D10S1221-D10S1432. The GAIN sample represents the largest series of asthma families investigated via genomic screening to date. The enhanced power associated with the increased sample size has led to the identification of several genomic regions that may be associated with asthma and/or asthma-related phenotypes.

A Genome-Wide Scan for Human Essential Hypertension Susceptibility Genes. *L.R. Griffiths¹, M.P. Johnson¹, R.A. Lea¹, S. Rutherford^{1,2}, R.P. Curtain¹, S. Hooker¹, C. Hutchins¹, D.R. Nyholt^{1,3}, M. Galley⁴, P. Kelly⁴, P. Reed⁴ and Genomics Research Centre, School of Health Science, Griffith University, Southport, Australia.* 1) Genomics Research Centre, School of Health Science, Griffith University, Southport, Australia; 2) Department of Pathology, University of Virginia Health System, Charlottesville, Virginia (present affiliation); 3) Genetic Epidemiology Laboratory, Queensland Institute of Medical Research, Royal Brisbane Hospital, Herston, Brisbane, Australia (present affiliation); 4) Gemini Genomics Pty Ltd.

Although essential hypertension is a major contributor towards human cardiovascular morbidity and mortality within Western populations, its aetiology has still not been clearly defined. It is clear that hypertension is a complex multifactorial trait involving both environmental and genetic components. In addition, hypertension tends to cluster with a number of related conditions including insulin resistance, obesity, dyslipidemia and diabetes, a disorder collectively termed metabolic syndrome or syndrome X. This report presents findings of a hypertension genome wide linkage scan in a Caucasian affected sib-pair cohort totalling 177 sib-pair comparisons. Single marker data using a two locus disease model provided significant evidence for linkage to a region on chromosome 20q (LOD 3.98, $P = 0.00002$) and evidence suggesting linkage to two other genomic regions (LOD 2.61, $P = 0.0005$ and LOD 3.22 $P = 0.0001$). Interestingly, a previous study has reported the same 20q locus (D20S107) in an independent obesity genome scan and in addition this region has also been implicated in diabetes. It is possible that this chromosome 20q region may harbour a major susceptibility gene that plays a role in the development of the complex phenotype associated with Syndrome X.

Linkage analysis and candidate gene investigation in osteoarthritis. *T. Forster, K. Chapman, L. Marcelline, J. Loughlin.* University of Oxford, Institute of Musculoskeletal Sciences, UK.

Knowledge about the pathways and mechanisms involved in the initiation and progression of osteoarthritis (OA) is sparse. By identifying genes conferring susceptibility to OA further insights in to this complex disease will be gained.

A previous genome-wide linkage scan of 481 families, each having at least one affected sibling pair, by our group identified five chromosomal regions that are likely to contain susceptibility genes, on chromosomes 2, 4, 6, 11 and 16.

To date finer linkage mapping, using polymorphic microsatellite markers at an average density of one marker every 3.3 cM in a total of 571 families, has allowed the regions on these chromosomes to be narrowed. Finer mapping of chromosome 16 has confirmed the existence of two separate susceptibility loci on this chromosome with maximum multipoint LOD scores (MLS) of 1.7 and 1.9. The first locus, which directly overlaps with a locus identified in a large Icelandic pedigree with primary, early onset hip OA, appears to be largely accounted for by affected female sibling pairs with hip OA. The second locus is more pronounced in affected female sibling pairs with hip or knee OA. A conditioning analysis indicates that these two chromosome 16 loci are interacting, suggesting that susceptibility resides in shared biological pathways.

Two loci have also been identified on chromosome 4. The first at 4q12-24 is accounted for by affected female sibling pairs. The second locus, identified in the total knee replacement strata, is located at 4q25-q35. This locus shows overlap with OA loci identified independently by two other groups. Both loci show a maximal MLS of 1.6.

Concurrently, single nucleotide polymorphisms (SNPs) in candidate genes within these regions have been identified and are being genotyped in cohorts of cases and controls. Genes targeted are those involved in the development, synthesis and maintenance of tissues within the joint and includes those encoding for the interleukin-4 receptor and xylosyltransferase I.

Genetic associations with cardiovascular risk and their dependence on sex. *J.A. Ellis, S.B. Harrap.* Physiology, University of Melbourne, Melbourne, Victoria, Australia.

Consideration of genotype-by-environment interactions in variance component based linkage analyses of quantitative traits is a well-established methodology. Although the idea has received relatively little attention, the interaction of genotypes with male and female *physiological* environments (genotype-by-sex) may be potentially important¹. This is especially true for diseases that display sex differences such as cardiovascular disease and also for genes relevant to sex determination. We have performed association analyses of several sex steroid related genes, including the genes encoding 5 α -reductase (*SRD5A1* and *SRD5A2*) and the estrogen receptors (*ERa* and *ERb*) with common risk factors for cardiovascular disease. Single nucleotide polymorphisms were genotyped in healthy Caucasian men (n = 386) and women (n = 332) from the general population of the Victorian Family Heart Study². Using ANOVA with adjustments for age and BMI, associations were sought with systolic blood pressure (SBP), diastolic blood pressure (DBP) and plasma fibrinogen in all subjects with adjustments for sex, and in males and females separately. No associations were demonstrable in the combined analyses. However, several associations were noted in males or females alone. The involvement of these genes in cardiovascular risk is currently being further tested using transmission disequilibrium tests and sib-pair linkage analyses. However, the results raise the possibility that mathematical adjustment for sex might be inappropriate if phenotypic effects of genotype are predicated on the sexual phenotype. Failure to consider genotype dependence on sex in genetic analyses of cardiovascular risk and other sex-relevant conditions may obscure contributing loci.

¹ Towne B, Siervogel RM, Blangero J. Effects of genotype-by-sex interaction on quantitative trait linkage analysis. *Genetic Epidemiology* 14:1053-1058, 1997. ² Harrap SB, Stebbing M, Hopper JL, Hoang HN, Giles GG. Familial patterns of covariation for cardiovascular risk factors in adults. The Victorian Family Heart Study. *American Journal of Epidemiology* 152:704-715, 2000.

Joint-analysis of two genome-wide scans conducted in Finnish study samples in schizophrenia and bipolar disorder. *J. Ekholm^{1,3}, M. Kestilä¹, J. Ekelund¹, T. Kieseppä², T. Paunio¹, P. Haimi¹, T. Varilo¹, M. Perola^{1,3}, T. Partonen², J. Lönngqvist², L. Peltonen^{1,3}.* 1) Department of Molecular Medicine; National Public Health Institute, Helsinki, Finland; 2) Mental Health and Alcohol Research; National Public Health Institute, Helsinki, Finland; 3) Department of Human Genetics; University of California Los Angeles, Los Angeles, USA.

We have carried out joint linkage analysis in families ascertained for schizophrenia or bipolar disorder by combing the raw genotype data from two genome-wide scans. Our aim was to localize potential loci, predisposing to a broader psychotic phenotype and playing a role in both diseases. Two diagnostic categories were assigned prior to the statistical analysis of the combined data set; 1) families diagnosed with psychotic disorder (psy) and 2) families diagnosed with affective disorder (aff). The total study sample of 2579 and 2198 subjects respectively from 313 families were screened with 339 markers spanning the genome. Twenty-seven loci exceeded a two point $Z_{max} > 1.0$, four of them exceeding a Z_{max} of 2.0 on chromosomes 1q32 ($Z_{max} = 2.48$: aff), 4q32 ($Z_{max} = 2.62$: aff), 5q11 ($Z_{max} = 2.56$: psy) and Xq25 ($Z_{max} = 2.45$: psy). The sample ascertained for bipolar disease was significantly smaller (331 subjects, 40 families), however, all four loci except for 5q11 represented linkage peaks also in the genome scan for bipolar disease. We propose that these chromosomal regions represent putative candidate loci for psychosis generally, and that pooled data analyses of study samples ascertained for traditional psychiatric traits should be pursued for different trait components to characterize the molecular background of these complex disease entities.

Linkage and association analysis of candidate genes for diabetic nephropathy. *K. Gogolin Ewens¹, R.A.V. George¹, L.K. Southworth¹, F.N. Ziyadeh², R.S. Spielman¹.* 1) Dept of Genetics, Univ of Pennsylvania School of Medicine, Philadelphia, PA; 2) Renal-Electrolyte and Hypertension Division and Penn Center for Molecular Studies of Kidney Diseases, Dept of Medicine, Univ of Pennsylvania School of Medicine, Philadelphia, PA.

Familial clustering of diabetic nephropathy (DN) suggests the existence of susceptibility genes that contribute to this disorder. In order to identify these genes, we have analyzed 72 candidate genes for linkage and association with DN. Families were ascertained through a proband with diabetes and end-stage renal disease (ESRD). DN in diabetic sibs of the proband was defined by an elevated albumin/creatinine ratio (ACR>300 mg/mg) in two of three urine samples collected six weeks apart. Diabetic sibs were considered unaffected for DN if they had normoalbuminuria (ACR<30 mg/mg), had duration of diabetes >15 years, and were not taking any ACE (angiotensin converting enzyme) inhibitor or AGTR1 (angiotensin receptor 1) blocker medications. Microsatellite markers within 50 kb of each candidate gene and/or SNPs in the gene were genotyped in 60 parent-child trios and 18 multiplex families. Nominally significant TDT results were obtained at nine genes, including two reported previously by others: AGTR1 and ACE. In the AGTR1 region, Moczulski et al. (*Diabetes*,47:1164,1998) found evidence for linkage (but not association) with DN using two polymorphic markers, ACTA and D3S1308 (15 kb and 600 kb 3' from AGTR1, respectively). We found an elevated TDT at both markers (D3S1308 allele 7: 37 (65%) of 57 transmissions, $c^2=5.1$; ACTA allele 6: 15 (71%) of 21 transmissions, $c^2=3.9$). These results support a role for the AGTR1 gene region in DN. An association between the D allele of the insertion/deletion polymorphism in ACE and DN has been reported in some studies but not others. In our study, we found evidence for elevated transmission of the D allele to DN offspring (31 (67%) of 46 transmissions; $c^2=5.6$). Nominally significant results were also found with markers in or near AGER, AQP1, COL1A1, HSGP2, PRKCB, TIMP3 and TGFBR3. However, in view of the small sample size in this study, these results must be considered preliminary.

Haplotype association analysis of bone mineral density and chromosome 1p36 candidate genes. *M. Devoto*^{1,2}, *H. Li*¹, *H. Rodriguez*³, *M. Koch*³, *H.S. Tenenhouse*⁴, *A. Tenenhouse*⁵, *L.D. Spotila*³. 1) Dept. of Research, Nemours Children's Clinic, Wilmington, DE; 2) Dip. di Oncologia, Biologia, e Genetica, Universita' di Genova, Italy; 3) Dept. of Bioscience and Biotechnology, Drexel University, Philadelphia, PA; 4) Dept. of Pediatrics and Human Genetics, McGill University, Montreal, Canada; 5) Metabolic Bone Centre, Montreal General Hospital, Montreal, Canada.

Osteoporosis is a common complex trait, characterized by reduced skeletal strength and increased susceptibility to fracture. Low bone mineral density (BMD) is the most important risk factor for osteoporosis, and several studies have confirmed the importance of genetic factors in determining variability of BMD. We have identified a candidate region for BMD in a sample of 42 families of individuals with low BMD. Our maximum multipoint lod-score by variance component linkage analysis was 3.53 for linkage of femoral neck BMD to a QTL located near marker D1S214 on 1p36. The empirical p-value by simulation analysis was equal to 0.0001. Two candidate genes located within the chromosomal interval 1p36.2-1p36.3, TNFRSF1B and PLOD, have been scanned for single nucleotide polymorphisms (SNPs) within their coding and promoter regions. These two genes are separated by about 200 kb. We detected four SNPs within TNFRSF1B and six SNPs within PLOD that were present with a frequency of greater than 5% in a sample of 104 North American individuals of European descent. Significant linkage disequilibrium among these SNPs was observed over a large interval within both genes. Haplotypes were reconstructed by means of a Bayesian algorithm implemented in the program Haplotyper, and haplotype association to BMD was investigated by linear regression analysis. TNFRSF1B haplotypes showed a statistically significant association with femoral neck BMD ($p=0.01$), whereas PLOD haplotypes demonstrated a statistically significant association with spinal BMD ($p=0.04$). These findings are consistent with a role of chromosome 1p36.1-p36.2 in BMD variability, and support the hypothesis that variations in these or in other nearby genes are important in conferring susceptibility to osteoporosis.

Linkage disequilibrium analysis in a susceptibility region for Bipolar Affective Disorder on 8q24. *S. Cichon¹, J. Schumacher², R. Kaneva², A. Van Den Bogaert¹, T.G. Schulze³, D.J. Müller⁴, M. Gross⁵, H. Fangerau⁵, T. Becker⁶, C. Richter², S. Ohlraun⁷, M. Rietschel⁷, P. Propping², M.M. Nöthen¹.* 1) Dept Medical Genetics, Univ Antwerp, Wilrijk, Belgium; 2) Inst Human Genetics, Univ Bonn, Bonn, Germany; 3) Dept Psychiatry, Univ Chicago, Chicago; 4) Mental State Hospital Bonn, Bonn; 5) Dept Psychiatry, Univ Bonn, Bonn; 6) Inst Medical Biometry, Informatics, Epidemiology (IMBIE), Univ Bonn, Bonn; 7) Central Inst Mental Health, Mannheim, Germany.

A genome wide screen for linkage to bipolar affective disorder (BPAD) has suggested a new susceptibility locus on 8q24 (Cichon et al., 2001). Marker D8S514 gave a two-point LOD score of 3.62 and a GENEHUNTER-NPL score of 3.56 ($p=0.00029$). The positive linkage region is large and extends over 30 cM. In an attempt to narrow down the linked region, we have performed linkage disequilibrium (LD) analysis in a sample of 119 parent-offspring trios with BPAD using the Transmission Disequilibrium Test (TDT). Part of the triads (53) were extracted from the genome screen families, the other 66 triads were independent. In a first step, eight microsatellite markers were tested for LD that are located in the highest linked region (about 5 Mb). Individual markers and two-marker haplotypes were tested for LD. TDT analysis revealed two regions of interest: One is characterized by preferential transmission of two particular two-marker haplotypes (markers A-C, $p=0.008$; markers B-C, $p=0.035$). A second region is located more centromeric. A particular allele of maker D ($p=0.01$) and the two-marker haplotype D-E ($p=0.001$) were preferentially transmitted. To follow up this finding, we have isolated new polymorphic STR markers in the region which are currently being analyzed by TDT.

Case selection strategies for association studies in linkage candidate regions. *T.E. Fingerlin, M. Boehnke, G.R. Abecasis.* Univ Michigan, Ann Arbor, MI.

For complex diseases, disease-marker association studies generally are necessary to identify disease-predisposing variant(s) in a linkage candidate region. Typically, a subset of individuals from the original family sample is genotyped for these association studies. We compared 4 strategies for choosing members of affected sibships to be genotyped in follow-up association studies in terms of (a) the expected difference in disease allele frequency between cases and controls (ED) and (b) the power to detect a disease allele frequency difference between cases and controls. The 1st strategy is to choose one sib at random from each sibship. The 2nd is to choose from each sibship the sib that shows the most evidence for IBD allele sharing with the other affected sibs. Since we assume the region has been targeted due to evidence for linkage, the 3rd and 4th strategies mirror the 1st and 2nd except that sibs are chosen only from those sibships that provide evidence for linkage to the region, where we define evidence for linkage as having an S_{all} value greater than expected for that sibship size assuming no linkage. For a wide array of disease models, and for sibship sizes 2-5, we calculated ED for the 4 strategies, and the expected proportion of sibships included for the 3rd and 4th strategies. We found that for affected sibship sizes >2 , ED was always greater when the sib showing the most evidence for sharing with the other sibs was chosen compared to randomly choosing a sib, whether one sib was chosen from each sibship or only from those sibships showing evidence for linkage. For nearly all models consistent with a common complex disease, ED was greater when one sib each was chosen only from sibships showing evidence for linkage rather than from all sibships, whether sibs were randomly chosen or chosen based on sharing with other sibs. For most recessive models, the increase in ED by using the 3rd and 4th strategies resulted in equal or increased power to detect a difference between cases and controls compared to the 1st and 2nd strategies despite the smaller sample size; for other models, this generally was not the case.

A Second Generation Genomic Screen For Multiple Sclerosis. *Y. Bradford*¹, *N. Schnetz-Boutaud*¹, *J.B. Rimmler*², *J.R. Oksenberg*³, *S.L. Hauser*³, *S. Schmidt*², *L.F. Barcellos*³, *R.R. Lincoln*³, *M. Clanet*⁴, *G. Edan*⁵, *J. Yaouanq*⁵, *G. Semana*⁵, *I. Cournu-Rebeix*⁶, *M.-C. Babron*⁷, *B. Fontaine*⁶, *M.A. Pericak-Vance*², *J.L. Haines*¹. 1) Program Human Genetics, Vanderbilt Univ, Nashville, TN; 2) Duke University Medical Center, Durham, NC; 3) Univ Cal San Francisco, San Francisco, CA; 4) Centre Hospitalier Universitaire Purpan, Toulouse, France; 5) Centre Hospitalier Universitaire Pontchaillou, Rennes, France; 6) Groupe Hospitalier Pitie-Salpetriere, Paris, France; 7) INSERM Le Kremlin-Bicetre, France.

Multiple sclerosis (MS) is a chronic inflammatory disorder of the central nervous system with a complex etiology. While the contribution of the Major Histocompatibility Complex (MHC) to genetic susceptibility for MS has been long established with strong associations to the HLA-DR locus (primarily to the HLA-DR2 allele), identifying other risk genes has been problematic. Previous genomic screens have suffered from small sample sizes and have identified numerous different regions, few of which have overlapped. We have completed a genomic screen with ~360 microsatellite markers (~10 cM spacing) on 250 families with 354 affected sibpairs and 119 other affected relative pairs, one of the largest screening datasets to date. 21 markers (defining 17 regions on chromosomes 1q, 3q, 4q, 5p, 6p, 6q, 7p, 9q, 11p, 11q, 13q, 15q, 18p, 20p, and 21q) met our initial criteria of a lod score > 1.0 for the semi-parametric NPL(pairs) or parametric MLOD statistics. The strongest signals are near the HLA-DR locus in the MHC on chromosome 6p (MLOD>3) and on chromosome 1q (180 cM on the Marshfield map, MLOD=2.12). The regions on 5p, 6q, 11p, and 15q overlap with regions identified in at least one previous genomic screen. Detailed analyses of these data suggest that some regions may interact with the HLA-DR locus. This may help explain the previous inconsistencies among the genomic screens.

Sib-similarity and the 10p problem. *J.H. Edwards.* Biochemistry, Oxford University, Oxford, Oxon, England.

Mein et al (1998), working on IDDM, showed a same:different ratio of parental alleles in pairs of affected sibs of 248:167 (63% same, $\chi^2 = 15.8$, $p < 0.0001$) after selecting a subset based on prior expectations. Raw data from www.cimr.cam.ac.uk/todd Davies et al.(1994) showed a similar pattern on earlier data from the same laboratory. This segment has now been incriminated on strong evidence in leprosy, on substantial evidence in four genome trawls of schizophrenia and four other psychoses, and in Paget's disease, with a supporting cast from autism, asthma and normal families from France and Denmark. See www.medvet.angis.org.au/www/w10p Other segments showing consistent sib-similarity include 6q, with strong evidence in IDDM and schizophrenia, moderate evidence in several disorders and one normal variant, height. Chromosome 14, 19 and chromosomal arms 10q, 16p and 16q also show excess segregation. The loci inferred in autism and Crohn's disease lie between main peaks. Such similarities are to be expected in sib-pair analyses based on any sibships with two or more affected, rather than the far more numerous sibships with one or more affected, as advanced by Penrose in 1935. medvet.angis.org.au/wspr/sibpr.htm Excess sib-similarity unrelated to disease due to embryonic lethals is a likely cause of the high losses between conception and birth in humans. In addition preferential fertilisation between gametes that lack identical alleles at some loci is likely. The evolutionary advantages of this mechanism, common to most plants, is likely to be exploited in vertebrates. However the problem remains. The symmetrical segregational disturbance is more than could attributed to recessive lethals alone, and neither meiotic drive nor preferential fertilization is likely to show symmetry. There is some tendency to paternal excess. If deficiency polymorphisms are a common cause of recessive lethals this will confound SNP trawls as the ends will show complete allelic association.

Conserved noncoding sequences (CNSs) on human chromosome 5q31 and susceptibility to asthma. *J. Donfack*¹, *K. Frazer*², *C. Ober*¹. 1) Dept Human Genetics, Univ Chicago, Chicago, IL; 2) Perlegen Sciences, Santa Clara, CA.

Comparisons of DNA sequences between species can identify functional elements. A recent study comparing human, mouse, and dog sequences identified 13 CNSs on human chromosome 5q31, including the region encoding the Th2 cytokines, IL-4 and IL-13 (Dubchak et al, *Genome Res* 2000;10:1304). One of these CNSs, CNS-1, was shown to coordinately regulate expression of these genes in transgenic mice (Loots et al, *Science* 2000;288:136). This region on 5q has been linked to asthma and atopy, disorders of Th2 cytokine dysregulation, in many populations, including the Hutterites, a founder population (TDT $P = 0.0009$). The objective of this study was to survey the 13 CNSs for variation and to determine whether variation in this region was associated with asthma or atopy or could explain the evidence for linkage in the Hutterites. We identified 7 single nucleotide polymorphisms (SNPs) in 5 of the CNSs. No variation was detected in 8 CNSs, including CNS-1. Based on allele frequencies and patterns of linkage disequilibrium (LD), we genotyped 4 SNPs and evaluated their association with asthma and atopy in the Hutterites, and in case/control samples of African Americans and European Americans. None of the SNPs were individually associated with asthma or atopy in any of the populations. However, haplotypes composed of alleles in one CNS and an intronic SNP in *IL4* showed nonrandom transmission to children with atopy (Global TDT $c^2 = 12.93$; $P = 0.004$), but did not explain the original evidence for linkage in this region. These combined data suggest that although some CNSs contain important elements for the coordinate regulation of Th2 cytokines, they do not contain variation that influences disease risk among individuals, although our studies in the Hutterites suggest that they may be in LD with variation that underlies susceptibility to atopic diseases. Furthermore, the fact that the only CNS known to have regulatory properties (CNS-1) showed no variation suggests that noncoding regions that are highly conserved between species may also show reduced levels of polymorphism within humans, similar to coding regions that are also highly conserved. Supported by HL49596 and HL56399.

Significant Association of Haplotype Blocks in the Urokinase Type Plasminogen Activator Gene on Chromosome 10 with Ab42 and Late Onset Alzheimer's Disease. *N. Ertekin-Taner^{1, 2}, M. Tucker^{3,4}, J. Ronald², S. Younkin², L. Younkin², M. Hella², S. Jain², D. Fadale², A. Hackett², L. Scanlin², J. Kelly², M. Kihiko-Ehman^{3,4}, S. Edland⁵, W. Markesberry⁴, R. Petersen⁶, N. Graff-Radford⁷, M. Hutton², S. Estus^{3,4}, S.G. Younkin².* 1) Department of Neuroscience, Mayo Clinic, Rochester, MN; 2) Department of Neuroscience, Mayo Clinic, Jacksonville, FL; 3) Department of Physiology, University of Kentucky, Lexington, KY; 4) Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY; 5) Department of Epidemiology, Mayo Clinic, Rochester, MN; 6) Department of Neurology, Mayo Clinic, Rochester, MN; 7) Department of Neurology, Mayo Clinic, Jacksonville, FL.

We obtained linkage to a locus on chromosome 10 at 81 cM in 10 late-onset Alzheimer's disease (LOAD) pedigrees, using plasma Ab42 levels as a quantitative surrogate phenotype. Linkage to the same locus was obtained in a genome screen of LOAD sib-pairs, providing strong evidence for a novel locus on chromosome 10 that increases the risk for LOAD by elevating Ab. The urokinase plasminogen activator gene (PLAU), located within the 1-locus support interval of our linkage peak on chromosome 10, converts plasminogen to plasmin, which degrades Ab aggregates. We tested PLAU as a candidate LOAD risk gene. We evaluated single nucleotide polymorphisms (SNP) in PLAU for association with Ab42 and LOAD in 10 extended LOAD families and 2 LOAD case-control series. There was strong linkage disequilibrium between the PLAU SNPs. PLAU SNP haplotypes were significantly associated with plasma Ab42 levels in 267 members of the AD families ($p=0.016$). PLAU SNP genotypes were also associated with AD in the first case-control series ($p=0.012$), and this result was replicated in the second series ($p=0.004$). When the two series were analyzed together there was highly significant association ($p=0.002$). Collectively, these findings implicate specific PLAU SNP haplotypes as novel risk factors for LOAD. Importantly, PLAU SNP haplotypes did not account for the Ab42 linkage at 81 cM, suggesting the presence of another LOAD risk gene on chromosome 10 (see abstract by Younkin et al.).

HLA-Cw*0602 as the primary risk factor for psoriasis accounts for all other observations of association with psoriasis in the HLA class I region. *A.M. Bowcock¹, C. Helms¹, T.M. Hsu¹, J. Daw¹, K. Cao², M.A. Fernandez-Vina², A. Menter³, J.P. Rice¹, P.-Y. Kwok⁴, N.L. Saccone¹.* 1) Washington University School of Medicine, St. Louis, MO; 2) Naval Medical Research Center, Georgetown University, Kensington, MD; 3) Baylor University Medical Center, Dallas, TX; 4) University of California San Francisco, San Francisco, CA.

Psoriasis is a complex inflammatory disease of the skin with both genetic and environmental risk factors. Associations with alleles from the HLA class I region (now known as PSORS1) were initially described. Extensive linkage disequilibrium within this region has made it difficult to identify the susceptibility allele although Cw*0602 is historically strongly associated with disease susceptibility.

To evaluate the HLA class I region more closely, we investigated both family-based association of DNA markers with psoriasis as well as linkage disequilibrium across the HLA class I region in patients and controls. HLA-Cw*0602 was the most highly associated allele with psoriasis in family-based analyses (TDT p-value = 0.000016), confirming reports from some groups that this is indeed PSORS1. However, linkage disequilibrium (LD) in case versus control chromosomes fluctuated throughout the HLA class I region, making identification of PSORS1 difficult with this approach. We thus applied two-locus logistic regression models to the case-control data, pairing HLA-C with other loci in the associated class I region. These analyses revealed that HLA-C*0602 remained significant while the effects of alleles at the other loci were explained by variation at HLA-C. This observation provides strong evidence for HLA-Cw*0602 as the primary risk factor for psoriasis in the HLA class I region.

Evidence supporting a language QTL on 7q in autism. *M. Alarcón¹, D.H. Geschwind¹, T.C. Gilliam², A.G.R.E. Consortium³, R.M. Cantor-Chiu⁴.* 1) Neurology, UCLA School of Medicine, Los Angeles, CA; 2) Columbia Genome Center, Columbia University, New York; 3) Cure Autism Now, Los Angeles, CA; 4) Human Genetics, UCLA School of Medicine, Los Angeles, CA.

Autism is a neurodevelopmental disorder typified by deficits in language and social skills, and repetitive behaviors. We previously found evidence for a quantitative trait locus for language deficits, as measured by 'age at first word' on chromosome 7q35 (Alarcón et al., 2002) in a sample of 152 families from the Autism Genetic Resource Exchange. The multipoint linkage peak of a nonparametric analysis by Genehunter first localized the putative gene between markers D7S1824 and D7S3058, a 24 cM region starting at 149.9 cM. Fine mapping with 28 flanking markers further localized it to a 10 cM region between D7S1824 and D7S2426. In the same sample, 'age at first phrase' was not linked to this region, whereas 'repetitive and stereotyped behavior' appeared linked when the flanking markers were included in the analysis ($Z = 2.48$, $p = 0.007$). Here, we report the results of adding 114 families and 9 markers to these analyses. Linkage to 'age at first word' was supported: the peak for the putative gene contributing to 'age at first word' has been narrowed and covers a 5 cM region between D7S676 and D7S2511 ($Z = 2.9$, $p < 0.002$). Consistent with our previous results, 'age at first phrase' was not linked to 7q. However, the 'repetitive and stereotyped behaviors' linkage evidence to 7q was not supported after adding these additional families. Thus, these results provide additional evidence there is a QTL for 'age at first word' in autism on chromosome 7q.

Linkage disequilibrium mapping of a type 2 diabetes susceptibility gene on chromosome 1q21-q23 in Utah Caucasians, Pima Indians, and Old Order Amish. *S.C. Elbein^{1,2}, H. Wang¹, W. Chu¹, J.K. Wolford³, R.L. Hanson³, B.D. Mitchell⁴, A.R. Shuldiner⁴.* 1) Department of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR; 2) Central Arkansas Veterans Healthcare System, Little Rock, AR; 3) NIDDK, National Institutes of Health, Phoenix, AZ; 4) Department of Medicine, University of Maryland, Baltimore, MD.

Family linkage studies have implicated chromosome 1q23 as a type 2 diabetes (T2DM) susceptibility locus, with evidence for linkage in Utah Caucasians, Old Order Amish, Pima Indians, and others. To identify the susceptibility locus in this region, we initially typed 1029 SNPs over 15 Mb in pooled samples comprising approximately 100 cases (T2DM) and controls from Pima Indians, Utah Caucasians, and Old Order Amish, plus an additional Amish pool of 59 individuals with impaired glucose tolerance (IGT). Typing was performed commercially using MassARRAY MALDI-TOF mass spectrometry (Sequenom, Inc). Between 511 and 600 SNPs were both polymorphic and successfully typed in each population. Of these, 28, 39, 19, and 33 SNPs showed significant ($p < 0.01$) initial differences between controls and Pima T2DM, Utah T2DM, Amish T2DM, and Amish IGT cases, respectively. We retyped all pools in triplicate for 93 initially suggestive SNPs, and also typed 11, 26, and 19 SNPs in the individual samples comprising each Pima, Utah, and Amish pool. After retyping in pools, 5, 12, and 19 SNPs remained significant ($p < 0.05$) in Pima, Utah, and Amish pools. Individual typing generally concurred with the pooled confirmations ($r > 0.4$), but with some error. We confirmed 9 SNPs in the Utah sample ($p < 0.05$), but only 2 SNPs with $p < 0.01$. Among Amish pools, 9 SNPs were confirmed in individual samples, of which 5 had $p < 0.01$. When both ancillary candidate gene studies and SNPs from pooled typing were combined in the analysis, we found a cluster of significant SNPs in the region between 157 Mb and 158 Mb (April 2002 Santa Cruz freeze) in all 3 populations. Additional regions in one or more populations were found at 148 Mb ($p = 0.001$ in Utah Caucasians), and 168 Mb (Amish T2DM). Our results are consistent with more than one T2DM susceptibility locus on 1q21-q23.

Mapping genes influencing Type 2 diabetes risk and Body Mass Index in Japanese. *N. Iwasaki*¹, *N.J. Cox*², *Y. Wang*², *P.E.H. Schwarz*², *G.I. Bell*², *M. Saito*³, *N. Kamatani*³, *M. Honda*⁴. 1) Diabetes Ctr, Tokyo Women's Medical Univ, Tokyo, Japan; 2) Departments of Human Genetics, Medicine, and Biochemistry and Molecular Genetics, Chicago, USA; 3) Department of Rheumatology, Tokyo Women's Medical Univ, Tokyo, Japan; 4) Department of Diabetes, Shiseikai Daini Hospital, Tokyo, Japan.

We have carried out an autosomal genome scan search for genes contributing to the development of type 2 diabetes mellitus (T2DM) in Japanese. We also mapped the chromosomal location of genes affecting body mass index (BMI). The 256 affected sib pairs (368 type 2 diabetic siblings from 164 families) were used. We found 12 regions on nine different chromosomes that showed nominally significant multipoint evidence of linkage with T2DM (LOD score > 0.59, $P < 0.05$): chromosome 1, 29.9 cM from pter ($P=0.030$); chromosome 2, 169.6 ($P=0.016$) and 236.8 cM ($P=0.047$); chromosome 4, 104.9 cM ($P=0.028$); chromosome 5, 114.8 cM ($P=0.040$); chromosome 6, 42.3 cM ($P=0.037$); chromosome 8, 15.3 cM ($P=0.033$) and 93.3 ($P=0.046$); chromosome 9, 140.0 cM ($P=0.006$); chromosome 11, 131.6 cM ($P=0.035$); chromosome 17, 36.1 cM ($P=0.040$); and chromosome 21, 48.0 cM ($P=0.001$). We found 12 regions on nine chromosomes showing nominal multipoint evidence for linkage with log transformed BMI (lnBMI): chromosome 2, 167.9 cM ($P=0.008$) and 210.5 cM ($P=0.005$); chromosome 3, 185.7 cM ($P=0.018$); chromosome 4, 118.9 cM ($P=0.007$) and 145.6 cM ($P=0.022$); chromosome 5, 131.9 cM ($P=0.019$); chromosome 7, 7.4 cM ($P=0.008$); chromosome 10 ($P=0.010$); chromosome 15, 12.8 cM ($P=0.014$); chromosome 16, 30.0 ($P=0.005$); and chromosome 17, 47.8 cM ($P=0.010$) and 100.2 cM ($P=0.005$). One of the lnBMI-linked loci (chromosome 2, 167.9 cM) maps to a region showing linkage with T2DM suggesting that it may be a "diabetes/obesity" gene. Eight of the 12 regions showing linkage with T2DM have been listed as candidate regions in other genome scans. Similarly seven of the 12 regions with BMI and BMI-related traits in other studies. Thus, our results replicate findings in other studies and may indicate new regions of the genome containing genes that contribute to the development of T2DM or regulate body weight.

A genome-wide scan in 438 families with early-onset coronary artery disease. *E.R. Hauser¹, D.C. Crossman², C. Granger¹, J.L. Haines³, C.J.H. Jones⁴, V. Mooser^{5,6}, L. Middleton⁶, A.D. Roses⁶, M.A. Hauser¹, M. Pericak-Vance¹, J.M. Vance¹, W.E. Kraus¹.* 1) Duke Univ, Durham, NC; 2) Univ of Sheffield, Sheffield, UK; 3) Vanderbilt Univ, Nashville, TN; 4) Univ of Wales, Cardiff, UK; 5) Lausanne Univ, Lausanne; 6) Glaxo-Smith-Kline, Philadelphia, PA.

We performed a genome-wide scan in 1168 individuals from 438 families comprising 491 affected sibling pairs with onset of CAD before age 51 in males and age 56 in females. CAD was determined by history of MI, coronary revascularization procedure or positive functional test. To identify more homogeneous groups, we pre-defined 3 stratification variables: 1) presence of acute coronary syndrome in ≥ 2 members of the sibship; 2) absence of Type 2 diabetes in one or more affected sibs; and 3) presence of metabolic syndrome defined by the 75th%tile of age-gender specific triglyceride levels, 25th%tiles of gender-specific HDL levels and BMI <35. Genotypes were produced for 395 microsatellite markers (an approximate 10 cM map). We used a battery of single point linkage analyses, including non-parametric affected sib pair (Siblink), non-parametric nuclear family analysis (Genehunter and Genehunter-plus) and affecteds-only parametric analysis (Fastlink and Homog). We also performed multipoint linkage analysis using Siblink and Genehunter-Plus. Regions were targeted for follow-up analyses if they provided lod scores greater than 1 on at least two analyses from 1) two-point parametric analysis using Homog and Fastlink performed for each marker and 2) two-point non-parametric analyses using Genehunter-Plus (nuclear families) and Siblink (ASPs) or 3) multipoint analysis. The affected siblings had mean age of 42 for men and 46 for women, 64% had prior MI, 72% had ACS, 19% had diabetes, 18% had metabolic syndromes. We identified 3 regions in the overall genome scan (chromosomes 1 (lod=1.3), 3 (2.1) and 17 (1.6)), 4 regions for the ACS families (chr 1 (lod=2.2), 3 (2.7), 15 (1.4), 16 (1.6)), 1 region for the non-diabetic families (chr 3 (lod=1.7)) and 3 regions for the metabolic syndrome families (1 (lod=1.1), 7 (1.3), 17 (1.1)). The relevance of these findings will be discussed in context of other published genome screens for CAD.

Association and linkage analyses of rgs4 polymorphisms in schizophrenia. *V.C. Kodavali¹, M. Karoly^{1,2}, S. Prachi⁵, J. Wood¹, E. Lawrence³, T. Bhatia⁶, S.N. Deshpande^{6,7}, B.K. Thelma^{5,6}, R.E. Ferrell³, F.A. Middleton², B. Devlin^{1,3}, P. Levitt², D.A. Lewis^{1,4}, V.L. Nimgaonkar^{1,3,6}.* 1) Department of Psychiatry, University of Pittsburgh, Pittsburgh, USA; 2) Department of Neurobiology, University of Pittsburgh, Pittsburgh, USA; 3) Department of Human Genetics, University of Pittsburgh, Pittsburgh, USA; 4) Department of Neuroscience, University of Pittsburgh, Pittsburgh, USA; 5) University of Delhi South Campus, New Delhi, India; 6) Indo-US Project on Schizophrenia Genetics, New Delhi, India; 7) Dr. R.M.L. Hospital, New Delhi, India.

Gene expression analyses of postmortem cerebral cortex suggest that transcription of the Regulator of G-protein Signaling 4 (RGS4) is decreased in a diagnosis specific manner in subjects with schizophrenia (Mirnics K et al, 2001). We have shown using the TDT, significant transmission distortion in three samples ascertained independently in Pittsburgh, New Delhi and by the NIMH Collaborative Genetics Initiative at this locus (Chowdari K et al 2002). Among SNPs spanning approximately 300 kb, significant associations involved four SNPs localized to a 10 kb region at RGS4, but the associated haplotypes differed. Consistent with the TDT results, samples with affected siblings (NIMH, India) showed higher levels of allele sharing, identical by descent, at RGS4. When the US patients were contrasted to two population-based control samples, however, no significant differences were observed. We also examined US families with Bipolar I Disorder (BD1) probands. This sample showed a trend for transmission distortion and cases differed significantly from the population-based controls for the four-SNP haplotypes tested in the other samples. The transmission distortion is unlikely to be due to chance. To understand these disparate findings, we have identified several novel SNPs localized in a 30 kb region upstream to the RGS4 locus for further analysis in our sample. We are also re-sequencing genomic DNA from the post-mortem brain samples employed in the gene expression analyses.

Association and Linkage analysis of von Willebrand factor and Bipolar I Disorder. *Y. Lee¹, M. Miller¹, A. Hasset², B.Y. Lee--Kim¹, J.J. Kim¹, M. Haraczak¹, S. Ranade¹, J. Wood¹, R. Reddy¹, D.J. Kupfer¹, F. Bontempo², V.L. Nimgaonkar^{1,3}.* 1) Department of Psychiatry, University of Pittsburgh School of Medicine and Graduate School of Public Health, Western Psychiatric Institute and Clinic, Pittsburgh, PA, USA; 2) Department of Medicine(Division of Hematology), University of Pittsburgh School of Medicine and Graduate School of Public Health, Western Psychiatric Institute and Clinic, Pittsburgh, PA, USA; 3) Department of Human Genetics, University of Pittsburgh School of Medicine and Graduate School of Public Health, Pittsburgh, PA, USA.

We report on association and linkage analyses of bipolar I disorder(BD1) and schizophrenia(SZ), using three common polymorphisms of von Willebrand factor(vWF), the gene conferring susceptibility to von Willebrand disease(vWD). The polymorphisms included 1548T>C, 2365A>G and an MSP I polymorphism in intron 19. The study was motivated by observed co-segregation of vWD and a range of mood disorders in an extended pedigree. Among 91 cases with BD1 and their available parents, the Transmission Disequilibrium Test(TDT) revealed significant transmission distortion of two haplotypes, but not the individual polymorphisms. To understand the overall transmission distortion, a global test of association for all haplotypes encompassing these SNPs was conducted. This analysis suggested significant transmission distortion(TRANSMIT program chi square=9.9, 3df, p=0.019). No significant associations were detected when the cases were compared with unrelated, population-based controls. To test whether the TDT results were due to general transmission distortion, 93 cases with schizophrenia and their parents were also investigated. Significant transmission distortion was not detected. Our analyses suggest linkage and association at vWF with BD1, but the effect is small and replicate studies are required.

Genome wide screen for genes related to healthy aging (wellness) in the NAS-NRC twin panel cohort. *T. Reed¹, D.M. Dick¹, T. Foroud¹, S.K. Uniacke², W.C. Nichols²*. 1) Dept Medical & Molecular Gen, Indiana Univ Medical Ctr, Indianapolis, IN; 2) Div Human Gen, Children's Hosp Medical Ctr, Cincinnati, OH.

The National Academy of Sciences-National Research Council twin registry is comprised of white veteran male twins born between 1917 and 1927 and represents about 93% of the male twin births in the above decade. An epidemiologic/health information questionnaire (Q8) was mailed between September and November of 1998 to 4086 twin-pairs with known addresses and 908 men who had completed both previous health surveys, but whose brother had died. Eliminating those with invalid addresses or the death of the subject, 6109 of 8848 (69%) questionnaires were completed comprising 2061 complete pairs with a mean age of 74.3 years.

A trait termed wellness, defined as a lack of chronic disease (no heart attack, no coronary surgery or angioplasty, no stroke, no diabetes, and no prostate cancer), had a heritability estimate (h^2) of 0.53 (0.46-0.61). An even narrower definition (also including no hypertension) had a h^2 of 0.57 (0.52-0.63) but approximately halved the number of pairs concordant for wellness. Fraternal (DZ) twin-pairs from this cohort provide a unique population to search for genes related to healthy aging.

A 10cM genome screen was performed using DNA from 79 DZ twin-pairs concordant for either of the wellness definitions obtained from the Q8 responses. Multipoint linkage analysis identified markers on 10 different chromosomes with a maximum LOD score ³ 1.0. Some of the more interesting locations in this preliminary analysis include a region near the apolipoprotein E locus on chromosome 19, and a region on chromosome 4 in the vicinity of a locus reported to be linked in families with extreme longevity (*PNAS* 98:10505,2001). Analyses including additional concordant twin-pairs will be performed as well as more extensive genotyping within areas of interest from the genome wide scanning. (Supported by AG18736).

Evidence for Haplotype Sharing and Association in Obese Finnish Males on Chromosome Xq24. *E. Suviolahti¹, L. Oksanen^{1,2}, R.M. Cantor¹, M. Öhman^{3,4}, K. Kontula², L. Peltonen^{1,3}, P. Pajukanta¹.* 1) Dept of Human Genetics, UCLA, Los Angeles, CA; 2) Dept of Medicine, University of Helsinki, Finland; 3) Dept of Molecular Medicine, National Public Health Institute, Finland; 4) Dept of Internal Medicine, University of Michigan, Ann Arbor, MI.

Obesity has been established as a risk factor for several important health problems such as type 2 diabetes, coronary heart disease, hypertension and osteoarthritis. It has been linked to a region on chromosome Xq24 in our genome wide scan of 166 Finnish nuclear families. We fine mapped this 30 Mb region by genotyping 10 microsatellite markers and 25 single nucleotide polymorphisms (SNPs) for 8 positional candidate genes in an augmented sample of 218 obese Finnish sibpairs (BMI>30 kg/m²) and in a Finnish case-control sample of 117 obese cases and 67 controls. The SNPs were selected in genomic regions conserved between humans and mice to increase their potential functional significance. The genotyped microsatellites and SNPs were tested for linkage and used for constructing haplotypes. Analysis of marker haplotypes significantly restricted the region from the previous 30 Mb to 4 Mb, between the markers DXS8088 and DXS8067. Importantly, haplotype analyses revealed that the evidence for linkage emerged mainly from the obese male sibling pairs, suggesting a gender-specific effect for the underlying gene. We also genotyped SNPs in three additional positional candidate genes: angiotensin receptor 2, solute carrier family 6 member 14 and adenine nucleotide translocator 2 and tested them for linkage and association. Our data show preliminary evidence for an association with some of these SNPs and their haplotypes (p<0.006). To replicate these results and identify the causative variants, we are genotyping the associated SNPs in a Finnish sample of obese and lean individuals.

Restriction of the susceptibility locus for migraine with aura on 4q24. *M. Wessman¹, G. Oswell², M. Kallela³, M.A. Kaunisto¹, J.C. Papp⁴, P.J. Tikka¹, A. Korhonen⁴, E. Hämäläinen⁴, H.T. Harno³, M. Ilmavirta⁵, M. Nissilä⁶, E. Säkö⁶, H. Havanka⁷, M. Färkkilä³, L. Peltonen⁴, A. Palotie².* 1) Dept of Clinical Chemistry, University of Helsinki, Helsinki, Finland; 2) Dept of Pathology, UCLA, LA, CA; 3) Dept of Neurology, University of Helsinki, Helsinki, Finland; 4) Dept of Human Genetics, UCLA, LA, CA; 5) Dept of Neurology, Central Hospital of Central Finland, Jyväskylä, Finland; 6) Turku Headache Center, Turku, Finland; 7) Dept of Neurology, Länsi-Pohja Central Hospital, Kemi, Finland.

Migraine is a complex neurovascular headache disorder with substantial evidence supporting a genetic contribution. There are two main types of migraine: without aura, occurring in 85% of patients; and with aura (MA), occurring in 15% of patients. MA attacks are preceded or accompanied by transient focal neurological, usually visual, aura symptoms. We recently reported the first genome-wide scan showing statistically significant linkage (HLOD 4.2) with the marker D4S1647 (at 104.95 cM) for MA on 4q24 on 50 clinically well-defined Finnish MA families. In the genome scan the λ -value for HLOD was 0.23-0.5, suggesting that 23-50% of the families would be linked to this region. When analysing the next set of 30 Finnish MA families the results confirmed that about 30% of our families contribute to the linkage to this chromosomal area. This area has further been fine mapped with 31 additional multiallelic markers. Based on two-point linkage analysis, the most likely location of the putative predisposing gene is in an 8 cM region between the markers D4S2278 (at 100.75 cM) and D4S1591 (at 107.95 cM). None of the markers tested so far show evidence for association. The clinical phenotype of the 4q24 linked families is characterized by severe vascular headache preceded by typical migraine aura with multiple provoking factors and premonitory symptoms. Overall, the phenotype corresponds well with the current criteria for migraine with aura.

Further Evidence of Linkage between Parkinson Disease and Chromosome 2q. *N. Pankratz¹, W.C. Nichols², S.K. Uniacke², C. Halter¹, A. Rudolph³, C. Shults^{4,5}, P.M. Conneally¹, T. Foroud¹ and Parkinson Study Group.* 1) Indiana Univ Sch Medicine, Indianapolis, IN; 2) Children's Hospital Research Foundation, Cincinnati, OH; 3) Univ Rochester, Rochester, NY; 4) Univ California, San Diego, CA; 5) VA San Diego Healthcare System, San Diego, CA.

While a number of different chromosomal regions have been implicated in autosomal dominant or autosomal recessive Parkinson disease (PD), only the role of alpha-synuclein and parkin have been clearly delineated. Mutations in the alpha-synuclein gene appear to be limited to only a few families; however, mutations in the parkin gene have been more widely reported, particularly among individuals with earlier onset PD. To identify susceptibility genes contributing to the more common, later onset form of PD, families consisting of at least one pair of living siblings diagnosed with PD were recruited through 60 Parkinson Study Group (PSG) sites distributed throughout North America. A Diagnostic Checklist was completed by the neurologist with inclusion criteria consisting of clinical features highly associated with autopsy-confirmed PD and exclusion criteria highly associated with other non-PD pathological diagnoses. Responses on the Diagnostic Checklist were then used to classify study subjects as having verified PD or nonverified PD. A genome screen was previously completed using 400 microsatellite markers. Families with a positive lod score in the chromosome 6 region near the parkin gene were screened for parkin mutations. All families with parkin mutations were removed from the subsequent analyses that were designed to identify novel genes contributing to PD susceptibility. With this approach toward reducing genetic heterogeneity, we have previously reported a lod score of 1.9 near the marker D2S206, in a sample of 96 sibling pairs with verified PD (Pankratz et al. 2002). We have since collected an additional 54 verified PD sibpairs from 46 families. When these families were combined with the previously collected sample, the maximum lod score increased to 3.0 near the marker D2S206. Therefore, these results provided further evidence of a PD susceptibility gene at 2q35. Study supported by: NINDS NS37167.

Ordered subsets analysis in Alzheimer disease: Refined linkage to 9p and novel linkage to 2q and 15q. *W.K. Scott¹, K.K. Nicodemus¹, P.C. Gaskell¹, D.J. Hedges¹, S.N. Walters¹, G.W. Small², P.M. Conneally³, A.D. Roses⁴, E.R. Hauser¹, J.R. Gilbert¹, J.M. Vance¹, J.L. Haines⁵, M.A. Pericak-Vance¹.* 1) Duke Univ., Durham, NC; 2) UCLA, Los Angeles, CA; 3) Indiana Univ., Indianapolis, IN; 4) GlaxoSmithKline, RTP, NC; 5) Vanderbilt Univ., Nashville, TN.

Alzheimer disease (AD) is a complex disorder with a wide range of ages at onset (AAO) within and between families. Considering AAO as a covariate in genetic linkage studies may reduce genetic heterogeneity and increase the power to detect linkage. Ordered subsets analysis (OSA) includes continuous covariates in linkage analysis by rank-ordering families by covariates and summing lod scores until a maximum is found. We analyzed data from 336 markers (10 cM average spacing) from a recent genomic screen of 455 multiplex AD families. Families were ascertained by: 1) a collaboration of Duke, Vanderbilt, and UCLA; 2) the Indiana AD Cell Repository; 3) the NIMH AD Genetics Initiative. To determine if GENEHUNTER+ non-parametric linkage results varied by AAO, families were rank-ordered by increasing and decreasing mean and minimum AAO. Permutation tests were used to assess significance of changes in the lod score. Two regions identified in the previously reported genomic screen provided significantly stronger results (lod>3) when considering AAO: 1) At D19S246 (19q13, 10 cM from APOE) lod=3.4 (p=0.009) in 107 families with mean AAO<70; 2) At D9S741 (9p22), lod=3.7 (p=0.02) in 405 families with minimum AAO>60. In addition, three regions not detected in the overall genomic screen provided interesting results (lod>2): 1) At D2S1384 (2q31), lod=2.8 (p=0.015) in 35 families with a minimum AAO<60; 2) At D2S125 (2q37), lod=2.4 (p=0.025) in 47 families with mean AAO>80; 3) At D15S153 (15q22), lod=2.7 (p=0.0023) in 39 families with mean AAO>80.5 and at D15S1507 (2 cM from D15S153) lod=2.3 (p=0.0015) in 37 families with minimum AAO>79. Consistent with previous analysis, OSA identified the region around APOE as most strongly linked in families with AAO<70, and the region of linkage on 9p as a late-onset (AAO>60) locus. The identification of novel linkages on 2q and 15q in very late onset AD (AAO>80) merits further investigation.

Genomewide search for celiac disease susceptibility loci in a Finnish population confirms the role of the HLA locus and provides evidence for a locus on 2q. *J.D. Rioux¹, H. Karinen², K. Kocher¹, S. McMahon¹, P. Krätkäinen², E. Janatuinen², M. Heikkinen², R. Julkunen², J. Pihlajamäki², A. Naukkarinen², V-M. Kosma³, M.J. Daly¹, E.S. Lander¹, M. Laakso².* 1) Human Medical and Population, Genetics Center for Genome Research, Whitehead Inst, MIT, Cambridge, MA; 2) Department of Medicine, University of Kuopio, Kuopio, Finland; 3) Department of Clinical Pathology, University of Kuopio, Kuopio, Finland.

Celiac disease (CD) is strongly associated with the HLA-DQ2 alleles DQA1*0501 and DQB1*0201. However, this association only accounts for a portion of the genetic component of CD. Several non-HLA loci and a few non-HLA candidate genes (including CTLA4) that potentially contribute to CD susceptibility have been reported, but have not yet been confirmed. In this study, we aim to determine the role of the HLA locus in a CD population from Finland. We also attempt to identify non-HLA loci that contribute to disease susceptibility in this population. To accomplish these aims, we performed genomewide linkage analysis on 146 CD patients and 104 healthy relatives from 54 Finnish CD families typed for 306 microsatellite markers. Our genomewide scan identified two regions of significant linkage to CD: 6p and 2q23-32. Specific typing at the HLA locus showed significant association of the DQ2 alleles with CD, likely accounting for the linkage peak at 6p. Because the linkage peak on chromosome 2 encompasses the CTLA4 gene, we typed each individual from our CD families for the A/G polymorphism at position 49 of the CTLA4 gene, but did not detect association in this population. Our analysis confirms the HLA-DQ2 alleles play a role in CD and provides evidence that a locus on chromosome 2q, that is distinct from CTLA4, confers risk to CD.

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Development of a Tumor Suppressor Gene (p53, BRCA, APC, ATM, RAD51, Rb, pTEN) SNP and Mutation Panel using the Single-Base Extension ELISA (SureScore™). *P.N. Gilles, P.T. Lieu, Y. Zhang, M. Gleeson.* Dept Genomics, Invitrogen, Carlsbad, CA.

Tumor suppressor genes exhibit a crucial role in many biological functions including DNA replication, DNA repair, gene expression, apoptosis and tumor development. The aim of this study was to develop an easily accessible panel of reagents to detect polymorphisms and mutations in tumor suppressor genes without the need for specialized instrumentation. To this end, a panel of approximately 50 SNPs and mutations in the p53, BRCA1, BRCA2, ATM, APC, RAD51, Rb and pTEN tumor suppressor genes have been developed using the single-base extension ELISA. The panel consists of genetic variations, which identify common haplotypes, contribute to disease, or alter gene function. The single-base extension ELISA in 96-well format was chosen as one of the simplest and most effective means of SNP typing and does not require specialized instrumentation (SureScore™, Invitrogen). The assay relies on the inherent ability of DNA polymerase to incorporate the precise nucleotide on to a capture oligo bound to a single-stranded PCR fragment; whereby the robustness of antibody-enzyme conjugates allows for the extended reactions to be scored colorimetrically at the same reaction conditions. The SNP and mutation reagents have been validated using a publicly available panel of diverse DNA samples (Corriell Institute), cell lines and controls. The panel can determine the genotypes of tumor suppressor genes in samples with unique phenotypes and the SureScore™ kit gives flexibility to scientists to design assays to detect other genetic variations with minimal optimization. The kit includes access to a web-based primer design software and data analysis software. The format is well established in the SNP Consortium (Orchid), and has been successfully applied to a number of SNPs on blinded genomic samples. SureScore™ analysis of tumor suppressor genes will facilitate the elucidation of gene functions and complex disease processes (www.invitrogen.com/surescore).

Dense SNP map of IL1 gene cluster and end stage renal disease. *J.T. Bensen¹, C.D. Langefeld², G.A. Hawkins¹, L.E. Green², J.C. Mychaleckyj¹, C.S. Brewer¹, D.S. Kiger¹, S.M. Binford¹, C.J. Colicigno³, D. Allred¹, B.I. Freedman⁴, D.W. Bowden^{1,3}.* 1) The Center for Human Genomics; 2) Department of Public Health Science; 3) Department of Biochemistry; 4) Department of Internal Medicine, Wake Forest University School of Medicine, Winston-Salem, NC.

Evidence supporting an inflammatory component in the development of nephropathy, characterized by glomerular basement membrane thickening and mesangial expansion exists. Studies have demonstrated a significant association between variations in the interleukin-1 receptor antagonist gene (IL1RN) and diabetic nephropathy. A dense SNP map was constructed across a 360 kb region containing the interleukin-1 gene cluster (IL1A, IL1B and IL1RN) focusing on IL1RN. In total, 95 polymorphisms were confirmed or identified primarily by direct sequencing: in IL1A, 13 SNPs and 1 insertion/deletion (4 bp); in IL1B, 5 SNPs; 1 intragenic tetranucleotide repeat; in IL1F10, a novel gene, 6 SNPs; and within IL1RN, 1 VNTR (86 bp), 3 single base insertion/deletions and 65 SNPs. Polymorphisms were mapped to completed BAC and genomic sequence spanning this region. The 95 polymorphisms were typed in 443 case-control subjects; 95 Caucasian controls, 75 Caucasians with type 2 diabetes mellitus-associated end stage renal disease (DM-ESRD), 86 African American controls, 92 African Americans with DM-ESRD and 95 African Americans with non-DM-ESRD. Consecutive pairwise marker linkage disequilibrium (LD) was not strictly correlated with distance and ranged from $D=0.0079 - 1.000$ and $D=0.0521 - 1.0000$ in Caucasians and African Americans, respectively. In general however, a shorter window of LD among African American than Caucasian controls was observed (50 kb vs. 350 kb for similar levels of LD, respectively). In addition, single marker and 2 and 3-marker haplotypes (EM method) were used to identify association with ESRD in IL1A, IL1F10 and IL1RN. Among African Americans, 8 and 3 single markers were significantly associated ($P<0.05$) with non-DM and DM-ESRD, respectively. The strongest disease-associated markers were in the IL1A gene, an intron 5 SNP ($P=0.0015$) and an I/D in the 3-UTR ($P=0.0024$), among African Americans with non-DM-ESRD.

Human insertion/deletion (indel) polymorphisms. *N. Ghebraniou¹, C. Zhao¹, L. Ott¹, D. David¹, G. Marth², R. Sachidanandam³, J.L. Weber¹.* 1) Center for Medical Genetics, Marshfield Medical Research Foundation, Marshfield, WI; 2) National Center for Biotechnology Information, NLM, NIH, Bethesda, MD; 3) Cold Spring Harbor Laboratories, Cold Spring Harbor, NY.

Insertion/deletions (indels) comprise roughly 20% of all human DNA polymorphisms. Using primarily overlapping BAC sequences from public sources and The SNP Consortium sequences, we have assembled a collection of about 200,000 human candidate indel polymorphisms. The collection of indels is available from the Marshfield web site (<http://research.marshfieldclinic.org/genetics>), and includes roughly equal numbers of diallelic and multiallelic polymorphisms. The collection is searchable using sequence positions along each chromosome. To date, about 2,400 of the candidate diallelic indels and 12,000 of the candidate multiallelic indels have been confirmed and characterized to varying degrees. Recently, we have focused efforts on indels that have relatively small numbers of tandem repeats. Multiallelic indels based upon long runs of tandem repeats (STRPs or microsatellites) are relatively well known. However, polymorphisms based upon small numbers of tandem repeats have not been closely studied. We find that for any short repeat sequence, the number of sequences within the human genome increases dramatically and monotonically as the numbers of repeats within the tandem array decreases. We also find that the fraction of sequences which are polymorphic drops dramatically as the number of repeats decreases. Candidate polymorphic indel sequences with relatively few numbers of dinucleotide tandem repeats appear to largely fall into the following four categories: 1) Compound repeat sequences with adjacent runs of two different tandem repeats 2) Sequences with interruptions in the runs of tandem repeats 3) Sequences with alternating purines and pyrimidines and 4) Short but uninterrupted runs of a single tandem repeat. We have also studied a few long, uninterrupted runs of tandem repeats which show little or no polymorphism within human populations. These sequences may mark segments of the genome which have either unusually low mutation rates or which are under strong selective pressure.

Refinement of the dyslexia 3 locus (*DYX3*) on chromosome 2p15-p16 and mutation screening of candidate genes.

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Developmental dyslexia is defined as a specific and significant reading disability that affects children and adults who in the absence of other neurological deficits possess normal intelligence, cognitive skills and adequate schooling. There is evidence of a considerable genetic contribution to the trait, but it is likely to be complex and to involve more than one gene. Several genetic loci have been mapped on chromosomes 1,2,3,6,15 and 18. We previously identified a family in which dyslexia is inherited as an apparent monogenic, dominant trait. We used this family to map the disease gene to a small region on 2p15-p16 by linkage analysis. The *DYX3* candidate region was defined by extensive haplotyping between D2S2352 and D2S1337.

The family has been investigated further and additional family members have been genotyped for new microsatellite markers. New flanking markers defining a 3.5 cM region on the physical map are D2S2153 and D2S444. The *DYX3* locus consists of 4.3 Mb of DNA sequence and contains 17 known and putative genes. There are no obvious functional candidate genes in the region, and genes have been screened mainly based on their location. However, genes with high expression in the brain have been considered first. Analysis of three candidate genes, BC200, Neurexin and Calcineurin 3, using a combination of Southern blot and direct sequencing of exons and intron/exon boundaries have revealed evidence of polymorphisms that are common to affected and control individuals.

Identification of SNPs in the central areolar choroidal dystrophy region on 17p13.2. *H.H. Abdeen¹, G. Silvestri², A.E. Hughes¹*. 1) Medical Genetics, Queen's University Belfast, Belfast, N Ireland, UK; 2) Ophthalmology, Queen's University Belfast, Belfast, N Ireland, UK.

Central areolar choroidal dystrophy (CACD) is a macular degenerative disease causing visual loss in middle age. The causative gene maps in a large Irish family to a 2 cM interval on chromosome 17 p13.2. The region contains over 50 genes, several of which are expressed in retina and are positional candidates for CACD. Over 20 genes have been investigated so far and over 100 SNPs identified. These include an interesting cluster of genes believed to be involved in macular degeneration, including PYK2 N-terminal domain-interacting receptor 1 (NIR1), aryl hydrocarbon receptor interacting protein-like 1 (AIPL1) and two other proteins of unknown function FLJ10156 and KIAA00523. This gene cluster was particularly rich in SNPs with a few C-SNPs. No mutations were detected in the members of the CACD family. However, the SNPs we have identified may be valuable for association studies in age-related and other macular degeneration.

Association study between the polymorphism D104N of endostatin, an angiogenesis inhibitor, and diabetic retinopathy in the Brazilian population. *F.I.V. Errera¹, M.E. Rossi², D. Gianella², B. Oliveira³, V. Takahashi³, M.R. Passos-Bueno¹*. 1) Centro de Estudos do Genoma Humano-Instituto de Biociências, Universidade de São Paulo, Brazil; 2) Departamento de Endocrinologia, Faculdade de Medicina da Universidade de São Paulo, USP, Brazil; 3) Departamento de Oftalmologia, Faculdade de Medicina Universidade de São Paulo, USP, Brazil.

Retinopathy is an important microvascular complication of Diabetes and a major cause of blindness. There is a growing list of evidences suggesting that its manifestation depend both on genetic as well as environmental factors. Angiogenesis is the hallmark of retinopathy and depends on a balance between activators and inhibitory factors. Endostatin, a proteolytic fragment of collagen XVIII, has been shown to be a potent endogenous inhibitor of angiogenesis. Recently, we showed that the endostatin polymorphism D104N changes the charge of the molecule and is associated with an increased risk for development of prostate carcinoma (*Cancer Res.*, 2001, 61: 7375-7378). Collagen XVIII plays a critical role in the maintenance of the structure of the retina (*Hum Mol Genet.*, 2000, 9: 2051-2058) and is highly expressed in blood vessels; therefore, we have hypothesized that the polymorphism D104N might be associated with susceptibility to diabetic retinopathy. We analyzed this polymorphism by Single Nucleotide Primer extension (SnPe; Amersham-Biotech) genotyping in 128 diabetic patients with retinopathy (66= non-proliferative, 30=pre-proliferative and 32=proliferative). The frequency of this polymorphism in the total sample was 11,7% and in each group was 12% (8/66), 10% (3/30) and 12,5% (4/32) for non-proliferative, pre-proliferative and proliferative retinopathy, respectively. These frequencies were very similar to that previously found in our control population (12%; *Cancer Res.*, 61: 7375-7378). This is the first study with a polymorphism on angiogenesis inhibitor and these preliminary results show no evidence of association between the polymorphism D104N of endostatin and diabetic retinopathy susceptibility. Other cSNPs in endostatin or in other anti-angiogenic inhibitors should be tested. This work was supported by FAPESP, HHMI, PRONEX, CNPq.

Lack of association of a promoter polymorphism of interferon regulatory factor 2 (IRF-2) gene with psoriasis in

Japan. *N. Hosomi*¹, *K. Fukai*¹, *N. Oiso*¹, *A. Kato*¹, *T. Murakami*¹, *M. Ishii*¹, *K. Nakajima*², *S. Shibahara*³. 1) Dermatology, Osaka City University Graduate School of Medicine, Osaka, Japan; 2) Immunology, Osaka City University Graduate School of Medicine, Osaka, Japan; 3) Molecular Biology and Applied Physiology, Tohoku University School of Medicine, Sendai, Japan.

Interferon regulatory factor 2 (IRF-2) is a transcriptional regulatory protein which represses for the interferon-alpha/beta genes. In mice lacking IRF-2, the inflammatory skin disease very similar to human psoriasis develops spontaneously. In addition, IRF-2 gene is located at human chromosome 4q35 where a familial psoriasis susceptible locus has been mapped. Furthermore, intravenous injection of interferon alpha for the treatment of hepatitis C infection often cause worsening of psoriasis. Therefore, we analyzed the IRF-2 as a possible candidate gene for common psoriasis in Japanese population. The coding region of the gene was normal in 35 patients with psoriasis. Then, we analysed the promoter region of IRF-2 gene and identified a 535A/G polymorphism in the IRF-2 transcriptional promoter. Association study was done in patients with 76 cases of psoriasis vulgaris versus 102 normal controls. We also recruited 113 patients with atopic dermatitis as the second control group. The association study showed no statistical differentiation between psoriasis, atopic dermatitis and normal controls. Computer-aided transcription search predicted that upstream stimulatory factor (USF) should bind only to G allele of the promoter. In fact, supershift assays showed specific binding of both USF-1 and USF-2 to only -535G allele of the IRF-2 promoter. However, luciferase assay revealed no statistical difference of the promoter activity between allele A and allele G. Furthermore, cotransfection of the USF-1 expression vector failed to stimulate the promoter activity of our luciferase reporter system. From these results, we conclude that the 535A/G polymorphism in the IRF-2 promoter is not associated with psoriasis and atopic dermatitis.

An ancient SNP haplotype in the Fanconi anemia gene *FANCA* occurs in 5% of the human population. *O. Levrán, K. Pujara, S.D. Batish, A.D. Auerbach.* Lab. of Human Genetics & Hematology, Rockefeller Univ., New York, NY.

The Fanconi anemia (FA) proteins function in DNA damage surveillance and patients are highly predisposed to cancer. The *FANCA* gene (FA complementation group A; GenBank NM 000135; 16q24.3) is highly polymorphic with more than 100 SNPs described (<http://www.ncbi.nlm.nih.gov/SNP>; <http://www.rockefeller.edu/fanconi/mutate/>). SNP haplotypes have become promising markers for human genetic studies, with evidence for blocks of SNPs in close physical proximity that are descended from an ancient ancestral chromosome. We have studied all 43 exons and flanking regions of *FANCA*, and have identified a conserved SNP haplotype spanning at least 60 Kb of the gene. The haplotype consists of at least 24 SNPs (9 common and 15 rare), located in exons 13,14,16,22,26,30,37,38 and 40, and introns 6,8,18,19,20,21,22,23,31,32,34,39 and 42. Most of the exonic SNPs are silent changes, except for 1235C/T (A>V), 1927C/G (P>A) and 3982A/G (T>A). A "representative" SNP (e.g. IVS20+30insGT) can be used to detect the haplotype, which occurs in 5% of normal or FA chromosomes. Carriers of this haplotype represent different ethnic groups (Caucasian, Asian, Jewish and African), suggesting an ancient origin for this allele whose stability is especially surprising because of the highly polymorphic nature of *FANCA*. There was only one pathogenic mutation (3391A>G) on this variant haplotype that occurred in two patients with no known familial relationship; otherwise the pathogenic mutations on this ancestral SNP haplotype all appear to be unique mutations of independent origin. A high proportion of *FANCA* mutations are large heterozygous deletions that are not detected by PCR and sequence analysis. We have used the homozygosity for these rare SNPs to detect large genomic deletions in nine FA-A families in which only one or no mutation was originally found. Since this haplotype spans a large part of the gene, it has enabled us to identify the extent of the deletions by studying the segregation and homozygosity state of each SNP within a pedigree. This haplotype may be a part of a larger SNP block and may contribute to the current effort to describe the haplotype structure of the human genome.

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Association of SPINK5 gene polymorphisms with atopic dermatitis in the Japanese population. *A. Kato, K. Fukai, N. Oiso, N. Hosomi, T. Murakami, M. Ishii.* Dermatology, Osaka City University, Medical School, Osaka, Japan.

Netherton syndrome (NS) is an autosomal recessive disorder characterized by trichorrhexis invaginata (bamboo hair), congenital ichthyosiform erythroderma, and atopic diathesis. NS has recently been shown to be due to a defect in the SPINK5 gene, encoding LEKTI, a 15-domain serine protease inhibitor. SPINK5 is located on chromosome 5q31, and has been suggested to be a locus predisposing to atopy in general. Recently, coding polymorphisms in SPINK5 exons 13 and 14 have been reported to be associated with atopy, asthma, and atopic dermatitis (AD). Here, we have examined whether this is also true in Japanese AD patients. We characterized eight polymorphisms in SPINK5 exons 13 and 14 in 124 Japanese AD patients and 110 healthy controls. The polymorphisms we examined were IVS12-26C>T, IVS12-10A>G, 1103A>G (Asn368Ser, in exon13), 1156G>A (Asp386Asn, in exon13), 1188T>C (His396His, in exon13), IVS13-50G>A, 1258G>A (Glu420Lys, in exon14), and IVS14+19G>A. We found significant associations between seven of these polymorphisms and AD in Japanese patients, confirming the previous suggestion of association between SPINK5 and AD.

Relationship between endometriosis and polymorphism of detoxification enzymes *NAT2*, *GSTM1* and *CYP1A1* in a Korean Population. *H.-S. Lee*¹, *J.H. Jun*¹, *S.Y. Park*², *I.S. Kang*³, *K.S. Han*³, *M.K. Koong*³. 1) Laboratory of Reproductive Biology and Infertility; 2) Laboratory of Medical Genetics; 3) Department of Obstetrics and Gynecology, Samsung Cheil Hospital and Women's Healthcare Center, Sungkyunkwan University School of Medicine, Seoul, 100-380, 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. The relationship between endometriosis and genetic polymorphism of detoxification enzymes *N-acetyl transferase 2 (NAT2)*, *glutathione S-transferase M1 (GSTM1)*, and *cytochrome P450 (CYP) 1A1* was investigated in a Korean population. DNA was extracted and analyzed in patients with minimal/mild (group I; n=23) and moderate/severe endometriosis (group II; n=30) and unaffected female controls (n=26) of Korean origin, using polymerase chain reaction and restriction fragment length polymorphism (RFLP). For *NAT2*, RFLP was used to detect the wildtype (wt) and mutant (mt) alleles, enabling classification into slow (mt/mt) or fast (wt/wt or wt/mt) acetylation genotypes. For *GSTM1*, PCR was used to distinguish active (+/- or +/+) from null (-/-) genotypes. For *CYP1A1*, *MspI* digestion was used to detect the wildtype (A1A1), heterozygote (A1A2) or mutant (A2A2) genotypes. The frequency of *NAT2* fast-acetylation genotypes in patients of endometriosis (group I and II) was significantly higher than that of controls (91.3% and 96.7% vs 76.9%; P<0.05). The frequency of *GSTM1* active genotypes was significantly greater in group II than controls (63.3% vs 38.5%; P<0.05). No significant difference was observed between patients and controls in the frequencies of the *CYP1A1 MspI* polymorphism, A1/A1, A1/A2 and A2/A2, respectively. However, the combination of the *GSTM1* null mutation and the *CYP1A1 MspI* polymorphism (A1/A2 or A2/A2) had a tendency for increased risk of endometriosis. We found an association between endometriosis and the detoxification enzyme *NAT2* fast acetylator, *GSTM1* active genotypes, and *GSTM1* null genotypes and *CYP1A1 MspI* polymorphism. These data suggest that affected detoxification enzymes activity may be related to the pathogenesis of endometriosis.

Analysis of the Defensin Gene Family for DNA Sequence Variants. *G.A. Hawkins¹, B-L. Chang¹, S.L. Zheng¹, T.D. Howard¹, A. Turner¹, S.D. Isaacs², K.E. Wiley², D.S. Postma³, E.R. Bleecker¹, P.C. Walsh², D.A. Meyers¹, W.B. Isaacs², J. Xu¹.* 1) Internal Med, Ctr Human Gen, Wake Forest Univ Sch Medicine, Winston-Salem, NC; 2) Dept. of Urology, Johns Hopkins Med. Institutions, Baltimore, MD; 3) U. of Groningen School of Med., the Netherlands.

Defensins are a family of small cationic peptides that act as antimicrobial and cytotoxic agents. Located within the 8p23.1 region, each defensin gene consists of 2 or 3 exons that encodes a 3-5 kDa arginine-rich peptide with extensive disulfide cross links. Depending on the positions of the disulfide crosslinks, defensins can be divided into two subclasses. Expression of the a subclass occurs primarily in neutrophils and intestinal Paneth cell granules, while expression of the b subclass occurs in various tissues including the gastrointestinal tract, trachea, placenta, thymus, colon, lung, epithelia cells, eye, and the genitourinary tract, including the prostate.

In two separate studies, we have evidence for linkage of two complex diseases in the 8p21-23 region: prostate cancer and asthma. In both complex diseases, inflammation caused by infectious agents has been proposed as a potential factor in disease progression and severity. Since defensins could play a role in controlling the growth of infection agents in these diseases, the defensin genes were targets for a candidate gene analysis. We sequenced six of the defensin genes, DEFA4, A5, A6 and DEFB1, B2 and B3 in two specific study panels. The first panel consisted of 96 hereditary prostate cancer (HPC) probands, and the second panel consisted of 96 individuals composed of equal numbers of American Caucasians, Dutch Caucasians, African Americans and US Hispanics, with each population divided into 16 asthma cases and 8 controls. Sequencing has identified: 25 SNPs in DEFB1, (1 non-synonymous); 17 SNPs in DEFB2, (1 non-synonymous); six SNPs in DEFB3, (no coding); 21 SNPs in DEFA4, (3 non-synonymous); 12 SNPs in DEFA5 (2 non-synonymous); and 11 SNPs in DEFA6, (no coding). The most notable non-synonymous SNP was a non-conservative amino acid change Ala19Glu in DEFA4 (2 asthma cases, 1 asthma control, 2 HPC).

Linkage and association analyses suggest the interleukin-1 receptor antagonist contributes to susceptibility of ankylosing spondylitis. *J. Luo¹, J. Akey¹, W. Maksymowych², L. Jin¹, J. McClain³, J. Reveille³.* 1) Center for Genome Information, Department of Environmental Health, University of Cincinnati, Cincinnati, OH; 2) Department of Genetics, University of Alberta, Canada; 3) University of Texas Health Sciences Center, Houston, TX.

Ankylosing Spondylitis (AS) is a common complex autoimmune disease. While approximately 90% of AS patients are HLA-B27 positive, other genes clearly contribute to AS susceptibility. We have conducted linkage and association analyses with microsatellite and SNP markers on chromosome 2q to better understand the genetic factors that contribute to AS. Specifically, three linked microsatellites spanning approximately 8 cM demonstrated significant linkage to AS in 187 affected sib-pairs (D2S160, NPL = 1.78, $p = 0.036$; D2S340, NPL = 4.09, $p = 0.00002$; D2S373, NPL = 2.37, $p = 0.009$). In silico analysis of this region led us to select the interleukin-1 receptor antagonist (IL1RA) for further study. In total, 101 cases and 48 controls were completely sequenced for all exons of IL1RA resulting in the identification of 37 SNPs. Furthermore, one SNP in exon 5 and two SNPs in exon 6 showed a significant association to AS (exon 5, $p = 0.03$; exon 6 SNP1, $p = 0.0019$; exon 6 SNP2, $p = 0.03$). Collectively, our data strongly supports the hypothesis that IL1RA contributes to AS susceptibility.

Program Nr: 1713 from 2002 ASHG Annual Meeting

A streamline method for SNP discovery. *P. McEwan, C. Fujii, J. Ziauddin, J. Malek, K. McKernan.* Agencourt Bioscience, Beverly, MA.

Discovery of a dense set of SNP markers across the genome, particularly in coding and regulatory regions, is a necessary first step in elucidating the role of genetic variation in common disease and drug efficacy. The SNP Consortium has identified and placed greater than 2 million SNPs into publicly available databases. Unfortunately, these public SNPs are randomly distributed throughout the genome, and there is a low probability of having high density SNP markers in specific regions of interest. As a result, additional directed SNP discovery is often required for pharmacogenomic studies focusing on candidate genes suspected in playing a role in a particular disease. Agencourt Biosciences SeeSNP Discovery program addresses this growing need for high throughput and directed SNP discovery in candidate regions. To address this need, we have developed a genomic alignment and amplicon modeling algorithm for the automatic generation of PCR primers across coding neighborhoods (exons and putative regulatory regions). The system utilizes an integrated Oracle LIMs system and a 384-well laboratory pipeline that relies on a high throughput-resequencing strategy. Newly discovered SNPs as well as in silico mined publically available SNPs are mapped to the candidate coding neighborhoods. Five microlitre, 384-well PCR and 1/32nd X BigDye, 384-well sequencing provide affordable high-throughput SNP discovery. Currently, 30,780 SeeSNP reads can be generated daily.

Development of a PCR based method for the determination of the BclI RFLP in the glucocorticoid receptor gene.
C. Lara-Castro, C. Song, Y. Chen, S.M. Sell. Nutrition Sciences, University of Alabama at Birmingham, Birmingham, AL, USA.

Background. Glucocorticoids have been implicated in the pathogenesis of obesity. Abnormalities in the glucocorticoid receptor gene (GCCR) such as the BclI restriction fragment length polymorphism (RFLP) in intron 1 have been associated with visceral obesity. Genotyping for this polymorphism is currently performed using the Southern blot technique. By using bio-informatics and data available through the human genome project, we were able to determine the region where this polymorphism is present, and to subsequently develop a PCR-based technique for the genotyping of this polymorphism. **Objective.** To develop a PCR-based, automated method for the determination of the GCCR BclI polymorphism. **Reagents.** DNA was amplified by PCR using standard conditions and the following primers: forward primer (5' — GGT TTG GAT GTG TGA CTA GC —3'); reverse primer (5'— CAA TGC AAT CCA TTT GCA CAG C —3'). The 317 bp product obtained was incubated with BclI restriction endonuclease (New England Biolabs), electrophoresed and analyzed. **Conclusions.** The PCR-based automated technique for the detection of the BclI RFLP in the GCCR gene described here will support faster analysis for large, population-based studies, and therefore a better understanding of the role of this polymorphism in the pathogenesis of obesity. This work was supported in part by funds from NIH grant P30DK056336.

A high throughput fluorescent assay for the detection of glutathione S-transferase polymorphic alleles. *R. Scholl¹, M. Blandford², L. Ballard¹, J.B. Magee¹, S. Williams¹, M. Robertson¹, F. Ali-Osman, D.Sc.³, R. Lemons, M.D., Ph.D², C. Keller, M.D.^{2,4}.* 1) Genomics Core Facility, University of Utah, Salt Lake City, UT; 2) Division of Pediatric Hematology-Oncology, Department of Pediatrics, University of Utah, Salt Lake City, UT; 3) Section of Molecular Therapeutics, Department of Neurosurgery, University of Texas, MD Anderson Cancer Center; 4) To whom correspondence should be addressed.

Polymorphisms of glutathione S-transferase (GST) enzymes are correlated with altered risk of selected cancers, as well as altered response and toxicity from the treatment of cancer. In this poster we demonstrate a low cost fluorescent polymerase chain reaction (PCR) based high-throughput assay that will facilitate the widespread study of how differences between GST alleles correlate with risk and with outcome of therapy for a variety of diseases, including cancer. Our assay design has three advantages over existing high-throughput assays. First, our assay design does not require restriction endonuclease digestions of PCR products in order to detect length differences between GST polymorphic alleles, as is needed for existing assays of GSTM1, GSTM3, or GSTP1. Second, our assay design allows all analyses to be performed simultaneously in the same electrophoresis gel lane. Third, our assay design is the first comprehensive, high-throughput method of assessing all four clinically-significant GST polymorphic alleles. Using an alternative genotyping method (single nucleotide extension) developed in our laboratory, we have determined the sensitivity and specificity of our assay to be 85 - 90% and 97 - 100%, respectively, depending on the source of genomic DNA.

Submicroliter genotyping of diallelic polymorphisms in microtape. A. Yu¹, J. Chen¹, T. Rusch¹, K. Fieweger¹, J. Chudyk¹, M. Doktycz², S. Hicks², W. Dickinson¹, N. Ghebraniou¹, J.L. Weber¹. 1) Center for Medical Genetics, Marshfield Medical Research Foundation, Marshfield, WI; 2) Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN.

Using a homogeneous assay with allele-specific PCR and fluorescently-labeled molecular beacons, we have developed a simple, two-step genotyping process for human diallelic polymorphisms. Reaction mixtures within sealed microtape are first amplified via thermal cycling, and then scanned for fluorescence without further manipulation. We have applied this assay to both substitution and insertion/deletion (indel) polymorphisms. For the indels, it is relatively easy to select robust allele-specific PCR primers. Microtape is a continuous thin strip of plastic embossed with an array of wells. It is an attractive substrate for genotyping and for many other chemical reactions. Microtape is manufactured by transiently heating a section of the plastic strip and then punching the wells using a metal tool. Because these tools are relatively inexpensive, many different versions of the microtape with varying well density, spacing, and geometry can easily be produced and tested. Our latest version of microtape (v.8) has shallow, conical wells with total volume of 1.1 l. Version 8 has 384-well arrays with dimensions that match standard 384-well microtiter plates. Microtape also has a series of indexing holes along each edge which allow the tape to be easily translated through pipetting, sealing, and scanning instruments. We have built a series of instruments to transfer liquid into the microtape using metal pins, syringes, and especially jetting of liquid droplets. A fluorescent scanner has also been developed along with software for analyzing the resulting signals. With v.8 microtape, reaction volumes are 400-800 nl; it may ultimately be possible to reduce reaction volumes to 100-200 nl leading to additional savings in reagent costs.

Significant Association of Five Candidate Gene Polymorphisms with Type 2 Diabetes in the Samoans. *D.T. Smelser¹, G. Sun¹, X. He¹, R. Indugula¹, M. Wolujewicz¹, H-J. Tsai², J. Tufa³, S. Viali⁴, S.T. McGarvey⁵, D.E. Weeks², R. Deka¹.* 1) Dept of Environmental Health, University of Cincinnati, Cincinnati, OH; 2) Human Genetics and Biostatistics, University of Pittsburgh, Pittsburgh, PA; 3) Department of Health, Pago Pago, American Samoa; 4) Department of Health, Apia, Samoa; 5) International Health Institute, Brown University, Providence, RI.

Type 2 diabetes is a complex disorder involving an intricate interaction between numerous environmental and genetic factors. The trademark of type 2 diabetes is hyperglycemia, resulting from impaired insulin secretion and utilization. Genes involved in the pathway of glucose metabolism are prime candidates in diabetes research. We identified 15 candidate genes and genotyped a single nucleotide polymorphism (SNP) within each one to test for an association with type 2 diabetes. We studied: *PC-1*, *PPARG2*, *FABP2*, *HNF-1b*, *GCK*, *NPY*, *GYS-1*, *ADRB3*, *UCP1*, *ADRB2*, *IGF1R*, *UCP3*, *LEP*, *ACE*, *IAPP*. We also genotyped 3 polymorphisms within the *Calpain-10* (*CAPN10*) locus, which had been identified as a susceptibility gene in a Mexican-American population (Horikawa et al. 2000 *Nat Genet* (26) 163-175). The Samoan islands are divided into American Samoa (AS) and Samoa (S). The independent nation of Samoa maintains a traditional Polynesian lifestyle, while AS has been culturally influenced by the U. S. Our cases consisted of 299 affected putative sib pairs and 1 half-sib pair from AS. The 96 unrelated controls were drawn from both AS and S. Five of the SNPs were found to be significantly associated using Fisher's exact test: *HNF-1b*, *IGF1R*, *UCP1*, *GCK*, and *ADRB3*. The locus *IGF1R* was in Hardy-Weinberg disequilibrium for the cases and controls, and for the cases only for *FABP2* and *ADRB2*. Haplotype frequencies and odds ratios were estimated for the *CAPN10* SNPs. No significant difference between cases and controls was found. Nonparametric linkage analysis was performed utilizing the *CAPN10* SNPs as well as 30 microsatellite markers spanning chromosome 2; the LOD score was 0. We are testing other candidate genes as well as additional SNPs in the above loci. (Supported by NIH grants AG09375, HL52611, DK55406 and DK59642).

A Genome-wide SNP Panel for use in Mapping and Association Studies. *K.E. Scott¹, J.F. Studebaker¹, F. Kuo¹, M.S. Phillips¹, P. Bauer¹, W.A. Ankeney¹, M.A. Donaldson¹, L. Cardon², M.T. Boyce-Jacino¹.* 1) Orchid BioSciences, Inc., Princeton, NJ; 2) Oxford University, Oxford, England.

Common diseases such as heart disease, cancer, diabetes, or asthma believed to be the result of complex polygenic phenotypes rather than a single gene variation. The “common variant — common disease” theory proposes that patterns of genetic variations frequently found in the human genome probably contribute to these common diseases. Therefore, identification of these patterns of variation may allow us to uncover the genetic factors of common diseases.

We have compiled a low-density panel of SNP markers that can be used to scan the genome for common genetic variants. The panel was designed for use with tag-array SNP-IT primer extension technology. The SNP-IT system provides a robust and highly reproducible assay with a more simple protocol for collecting genome-wide genotypes than the commonly used microsatellite mapping process. Automated SNP-IT technology using the SNPstream UHT instrumentation allows for more than 100 unique genome-wide scans to be collected in a single day. Additionally, the entire scan can be conducted using as little as 500 ng of genomic DNA. By comparison, microsatellite scans require microgram quantities of DNA, take weeks to run, and achieve spacing of only approximately 10 Mb.

The panel of SNPs provides coverage of all characterized regions of the human genome at an average of 1 Mb spacing. SNPs were selected based on map location and allele frequency in three diverse populations in order to achieve this coverage. We will present data demonstrating the utility of our genome-wide panel by comparing the data we have collected from the panel to that of the microsatellite set.

Sequence Variation in the *WFS1* Gene. T.A. Sivakumaran¹, J.C. Long^{2,3}, M.M. Lesperance¹. 1) Otolaryngology-Head & Neck Surgery, University of Michigan, Ann Arbor, MI; 2) Department of Human Genetics, University of Michigan, Ann Arbor, MI; 3) Center for Statistical Genetics, University of Michigan, Ann Arbor, MI.

Mutations in the Wolfram syndrome 1 gene (*WFS1*) cause both autosomal recessive Wolfram syndrome and autosomal dominant low frequency sensorineural hearing loss (LFSNHL) linked to DFNA6/14/38. Several different heterozygous missense mutations in *WFS1* have been reported to underlie LFSNHL, whereas the majority of the mutations in Wolfram syndrome are homozygous inactivating mutations. In addition, several polymorphic variants in *WFS1* have been reported, some of which are associated with diabetes mellitus and/or psychiatric disorders. With a large number of nonsynonymous variants, it becomes difficult to determine if any given variant is disease-causing versus a benign polymorphism. No study to date has attempted to look at the entire coding sequence in a large number of controls reflective of the U.S. population. We sequenced the entire coding (2673 bp) and the flanking intronic region (463 bp) of *WFS1* in 96 control individuals, of which 90 were from the DNA Polymorphism Discovery Resource. A total of 49 simple sequence variations were found. 42 were in the coding region, and most (35/42) were in exon 8, by far the largest exon in *WFS1*. 41/42 were base substitutions (53.6% synonymous). About half of the variations were found only once in 192 chromosomes screened. The observed genotype frequencies of these variants were within Hardy-Weinberg equilibrium (HWE), except for R611H. The deviation ($p < 0.05$) from HWE for R611H is due to a lack of heterozygotes, which is not surprising in an admixed population. The nucleotide diversity coefficients (p) were 13.37×10^{-4} and 10.28×10^{-4} for the coding and non-coding regions, respectively. Tentative haplotype assignments constructed from 13 single nucleotide polymorphisms (SNPs) revealed 23 different haplotypes, with five haplotypes accounting for 83% of chromosomes. Linkage disequilibrium analysis of *WFS1* showed strong disequilibrium over the entire genomic sequence. The results have important implications for the use of these SNPs in the genetic analysis of families with LFSNHL as well as for association studies of other disorders.

Evidence for an association between IL-9 polymorphism and susceptibility to Visceral Leishmaniasis. *H. Elloumi Zghal¹, S. Abdelhak¹, A. Ben Salah², M.R. Barbouche¹, M. Ben Fadhel¹, A. Garnaoui¹, K. Dellagi¹.* 1) Immunology, Pasteur Institute, Tunis, Tunisia; 2) Ecology and Parasitic Epidemiology, Pasteur Institute, Tunis, Tunisia.

Mediterranean Visceral Leishmaniasis (MVL) is caused by *L. infantum*, an obligate intracellular parasite of mononuclear phagocytes that mainly affects children under 5 years old. Experimental Leishmaniasis in inbred mouse strains as well as segregation studies have shown that genetic factors may affect the susceptibility to Visceral Leishmaniasis but no specific human genes have been identified. Ten candidate genes have been selected on the basis of their relevance to disease pathogenesis and the presence of intragenic polymorphisms of possible biological significance. To investigate the role of these polymorphisms in susceptibility to MVL, we have carried out a case control study in a sample of 103 patients with MVL and 105 ethnically matched unrelated healthy controls, selected on the basis of a positive Leishmanin Skin Test, from an endemic region in central Tunisia. Allele frequencies were compared with the use of a two by- two chi-square test. We have not observed any evidence of association between susceptibility to MVL and the NRAMP1 gene, which has been reported to regulate the early resistance and susceptibility infection with *Leishmania* in mouse strains. Analysis of an intragenic IL-9 polymorphism showed a strong association of the allelic variant 124 and susceptibility to MVL ($\chi^2=9.79$ with 1 df; $p=0.0017$). In the other hand, a protective effect of the 142 allele of a polymorphism in the IL-10 promoter was noted in the studied sample ($\chi^2=4.04$ with 1 df; $p=0.044$). This is to our knowledge, the first description of an association between IL-9 gene and Visceral Leishmaniasis. IL-9 is a member of the Th2 cytokine family that has recently been involved in the pathology of human asthma as well as in the response to parasitic infections, and inflammatory processes. Our results indicated that IL-9 may be directly involved in LVM susceptibility, although, the association observed may be the result of strong linkage disequilibrium between IL-9 gene and another nearby susceptibility gene.

Genetic of bitter taste: identification of a Ch 7q35 locus in a Sardinian genetic isolate. *D.A. Prodi¹, D. Drayna², A. Angius¹, P. Forabosco¹, D. Piras¹, M.A. Palmas³, M. Pirastu¹.* 1) Istituto di Genetica delle Popolazioni, CNR, Alghero, SS, Italy; 2) National Institute of Deafness and Other Communication Disorders, Rockville, USA; 3) Sharda, Cagliari, Italy.

The inability to taste phenylthiocarbamide (PTC) is one of the best studied simple inherited traits in humans, but genetic linkage studies of this trait have produced inconsistent and conflicting results. Recently, a linkage study found a major locus on chromosome 5p15 and evidence for an additional locus on chromosome 7q. We sought to clarify these studies by using an exceptional genetic isolate residing on the island of Sardinia. This population consists of approximately 1200 individuals, 85% of which are descended from fewer than 20 founder couples. Phenotypes were determined using six scalar solutions of PTC with scores corrected for age and sex. Qualitative PTC taste threshold assignment in 280 individuals gave 75% tasters and 25% non tasters in this population. We tested for linkage at 400 microsatellite markers spaced across the genome using 110 selected informative individuals. Parametric and non-parametric analysis, performed with LINKAGE and GENEHUNTER, showed significant values at 7q35 (peak two-point lodscore of 3.03 at D7S661 and multipoint lodscore of 2.77). Phenotype was also analyzed as a quantitative trait using SOLAR which confirmed localization on chromosome 7: we obtained a two-point lodscore of 4.31 and a multipoint lod score of 4.73 in the same region. No strong evidence for linkage was obtained on chromosome 5p (multipoint lod score of 1.36) These results support previous studies assigning linkage of PTC tasting to the region surrounding the Kel blood group antigen on 7q.

Allele frequency determination of a 10 cM clustered set of SNPs for the TSC Linkage Map project. *J.D. Winick, A. Silbergleit, W. Lee, B. Chui, M.R. Bonner, P. Gwynne, M. Gaskin, A. Gandhi, T. Perez, M. Hernandez, J. Zdunek, A. Ledesma, S. Lew, R. Shippy, P. Stafford, J. Goldsmith, K. McWeeny, C.T. Yamashiro.* Motorola Life Sciences, Tempe, AZ.

In collaboration with The SNP Consortium (TSC), we have determined allele frequencies for a 10 cM SNP cluster map set using the CodeLink SNP genotyping platform. A SNP cluster is defined as a selection of SNPs from the total content contained in a 100 kb genomic region. Each SNP cluster is linked to a Marshfield microsatellite, which was used to determine an approximate average of 10 cM spacing. The TSC Linkage Map project was set up in two phases. The first phase was to determine the allele frequencies of candidate SNPs within three diverse population sample sets. The second was to use this data to select SNPs to be used to genotype 50 CEPH pedigrees to produce a SNP based linkage map. In all 2222 SNPs from 335 SNP clusters were evaluated for Phase I. Of these, 1517 passed the CodeLink primer and probe design process. One to four SNPs (1024 total SNPs) within 331 of the 335 SNP clusters were selected for evaluation on the 90 individuals from the TSC diversity panel (30 Caucasians, 30 African-Americans and 30 Asians). Minor allele frequency was determined for 851 of the 1024 SNPs evaluated, of which 740 or 87% of SNPs analyzed were polymorphic in at least one population and 606 SNPs were polymorphic in at least 2 out of 3 populations tested. It was found that in 2 of 3 of the test populations, 62.9% of SNPs had a minor allele frequency of >10% and 49.6% >20%. 106 SNPs proved to be monomorphic in all 3 populations tested. Each SNP was sequence confirmed for accuracy against three diverse DNA samples and only accurately performing SNPs progressed in the analysis. A total of 76,590 genotypes were reported in the minor allele frequency determination phase of the project. All 797 successfully designed and functioning SNPs, regardless of polymorphic state, were genotyped in 50 CEPH pedigrees (~640 samples and ~510,000 genotypes) for phase II of the TSC linkage-mapping project and all informative SNPs were included in the map.

Program Nr: 1723 from 2002 ASHG Annual Meeting

Do we need to correct for population stratification in the UK BioBank initiative? *M.P.H. Stumpf, N. Soranzo, D.B. Goldstein.* Department of Biology, University College London, London, UK.

We present the results of a large-scale study of population stratification in Northern Europe. We have genotyped over 1 000 males from throughout the British Isles and other parts of Northern Europe at 20 completely unlinked, autosomal SNPs to establish how population stratification in the UK is likely to affect the MRC/Wellcome Trust BioBank program. The SNPs were chosen to be at least 80kb from the nearest known gene or EST; thus we also expect them to be selectively neutral. With this set of SNPs it became possible to (i) assess the likely extent of stratification in North-European population samples and (ii) develop adequate methods for correcting for spurious association resulting from admixture in the UK. The experimental study was complemented by extensive use of coalescent simulations of an N-island demography with migration. This allows us to directly assess divergence of populations in the UK that may cause problems in large-scale case-control studies. We find that spurious associations can be a concern in the UK, especially in extremely admixed population samples (generated, for example, by pooling the geographically most separated sub-populations in our study). Even if there are detectable levels of stratification they are not always high enough to reliably group population samples into distinct sub-populations for further analysis. Thus we believe that standard methods of genomic control offer the most convenient method to correct for cryptic population structure in the UK. We were also able to confirm the statistical significance of the experimental results through computer simulations. In particular we show that the UK data is incompatible with high levels of population divergence between the regions in the British Isles. Moreover we provide evidence that the present set of 20 unlinked SNPs will be sufficient to keep the rate of false positives below the 5% level using standard methods of genomic control.

Association of GABA receptor rho1 gene polymorphisms with severe myopia. *E.C. Tan¹, S.H. Ng¹, S.H. Yap¹, L. Gan¹, U. Karupathivan¹, H.M. Wu¹, E.P.H Yap^{1,2}.* 1) Defence Medical Research Institute, Defence Science and Technology Agency, Singapore; 2) Singapore Eye Research Institute.

The etiology of human myopia is unknown. While environmental factors such as near work and educational attainment are important, family history and ethnic background are established risk factors suggesting a genetic predisposition to the development of severe myopia. In our ongoing study to identify susceptibility genes for myopia, we target retinally expressed genes as functional candidates. Among molecules involved in the retinal signaling process, GABA (gamma-aminobutyric acid) is the primary inhibitory neurotransmitter in the adult mammalian retina. There are at least three types of GABA receptors in the retina. For the r1 subunit which is one of the components of the GABA_A receptors, expression is primarily confined to retinal bipolar cells and cerebellum and is also developmentally regulated. In this study, we genotyped 2 single nucleotide polymorphisms in the r1 gene and carried out initial association analysis using 87 controls and 85 subjects with refractive errors of between -8.5 and -18.25D. Controls are ethnically, gender and age-matched subjects with refractive errors of less than -0.5D. For the T99C polymorphism, which involved an amino acid substitution, there was some difference in the distribution of the genotypes between the two groups but it did not reach statistical significance. The difference in genotype distribution was marginally significant for the C457T polymorphism ($\chi^2=3.73$, $p=0.053$). Difference in allele frequency was also marginally significant between cases and controls ($\chi^2=3.47$, $p=0.063$) with the odds ratio at 3.3 (95% CI: 0.88-12.04). Haplotype analysis was performed to obtain estimated haplotype frequencies by diagnosis group separately and for all subjects combined. There was no significant linkage disequilibrium between the 2 single nucleotide polymorphisms. There was also no significant difference in the distribution of the 4 possible haplotypes between cases and controls. We will confirm the positive association with a larger set of samples and also investigate other polymorphisms within this gene.

Fine Mapping Linkage Analysis of COPD-related Phenotypes on Chromosome 19. *J.C. Celedon^{1,2}, L.J. Palmer^{1,2}, B. Raby^{1,2}, J.M. Senter¹, J.M. Drazen^{1,2}, D.J. Kwiatkowski^{1,2}, H.A. Chapman³, M. Hernandez¹, F.E. Speizer^{1, 2}, S.T. Weiss^{1, 2}, E.K. Silverman^{1, 2}.* 1) Channing Laboratory, Brigham and Women's Hospital, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) University of California at San Francisco, San Francisco, CA.

Severe alpha 1-antitrypsin (AAT) deficiency is the only proven genetic risk factor for chronic obstructive pulmonary disease (COPD). To study novel genetic determinants of COPD, we enrolled 72 pedigrees ascertained through severe, early-onset COPD probands (FEV1 < 40% pred., age <53 yrs, without severe AAT deficiency). Results of a 10 cM genome scan with short-tandem repeat (STR) markers have been previously reported (AJHG 2002;70:1229-39). Following the genome scan, we conducted linkage analysis of 15 STR markers (5 from the original scan and 10 additional markers) on chromosome 19 (range, 33 to 68 cM) with COPD-related phenotypes in 585 members of families of the severe, early-onset COPD probands. We used SOLAR for variance component linkage analysis of quantitative traits (FEV1 and FEV1/FVC), and the NPL all statistic (in MERLIN) for the analysis of qualitative traits (chronic bronchitis, mild airflow obstruction [FEV1 <80% pred. and FEV1/FVC <90% pred], and moderate airflow obstruction [FEV1 <60% pred. and FEV1/FVC <90% pred). Compared with the genome-wide scan, there was increased evidence for linkage on chromosome 19 for FEV1, FEV1/FVC, and moderate airflow obstruction. In the original genome scan, the maximum multipoint LOD scores were: 1.40 (at 59 cM) for FEV1; 1.47 (at 61 cM) for FEV1/FVC; and 1.54 (at 42 cM) for moderate airflow obstruction. In the current analysis, the maximum multipoint LOD scores were: 1.73 (at 61 cM) for FEV1; 1.64 (at 62 cM) for FEV1/FVC; and 2.31 (at 36.2 cM) for moderate airflow obstruction. These results provide further suggestive evidence that there is a locus on chromosome 19 that contributes to airflow obstruction, an important COPD-related phenotype. This abstract is funded by NIH Grants: HL61575 (EKS) and NIH HL04370 (JCC).

Common functional polymorphisms in Toll-like Receptor 4 (TLR4) are not associated with asthma or atopy-related phenotypes. *B.A. Raby^{1, 2}, W.T. Klimecki³, C. Laprise⁴, Y. Renaud², J. Faith², M. Lemire², C. Greenwood², C. Lange⁵, L.J. Palmer¹, R. Lazarus¹, D. Vercelli³, D.J. Kwiatkowski¹, E.K. Silverman¹, F. Martinez³, T.J. Hudson², S.T. Weiss¹.* 1) Channing Laboratory, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; 2) Montreal Genome Centre, McGill University Health Centre, Montreal, QC, Canada; 3) Arizona Respiratory Center, University of Arizona, Tucson, AZ; 4) University of Quebec at Chicoutimi, Departments of Fundamental Sciences and Human Sciences, Chicoutimi, QC, Canada; 5) Department of Biostatistics, Harvard School of Public Health, Boston, MA.

Toll-like receptor 4 (TLR4) is the principal receptor for bacterial endotoxin recognition. Functional variants in the gene confer endotoxin-hyporesponsiveness. There is also evidence that endotoxin exposure during early life protects against the development of atopy and asthma. It is therefore possible that genetic variation in TLR4 contributes to asthma susceptibility. The purpose of this study is to characterize the genetic diversity in TLR4 and test for association between the common genetic variants and asthma-related phenotypes. In a cohort of 90 ethnically diverse subjects, we resequenced the TLR4 locus, identified a total of 29 single nucleotide polymorphisms (17 of which are novel) and inferred the haplotype structure. We assessed five common polymorphisms for evidence of association with asthma in two large family-based cohorts: a North-American cohort (589 families), and a founder population from northeastern Quebec, Canada (167 families). Using the transmission-disequilibrium test (GENEHUNTER 2.0) we found no evidence of association with asthma for any of the polymorphisms tested, including two functional variants D259G and T359I. We also found no evidence of association with the common TLR4 haplotypes. Furthermore, using FBAT, we found no evidence for association between the TLR4 variants and four quantitative intermediate asthma- and atopy-related phenotypes (FEV1, PC20, total serum IgE and serum eosinophils). Based on these results, we find no evidence that genetic variation in TLR4 contributes to asthma susceptibility. Grants: NIH HL07427 HR16049 HL66386 HL66795 CIHR: MC1-40745.

ASSOCIATION BETWEEN THE CA POLYMORPHISM IN INTRON 13 OF THE ENDOTHELIAL NITRIC OXIDE SYNTHASE GENE (eNOS) IN ISCHEMIC HEART DISEASE PATIENTS OF MEXICAN

POPULATION. *R.P. Mariaud¹, M.P. Gallegos¹, L. Arnaud¹, T.J. Beltran², M.R. Arechavaleta², G.M. Zuniga¹.* 1) Medicina Molecular, IMSS, Guadalajara, Jalisco, Mexico; 2) Departamento de Endocrinologia, HE, CMNO, IMSS, Guadalajara, Jalisco, Mexico.

INTRODUCTION. Recently several polymorphisms of the endothelial synthase gene have been associated as risk factors for ischemic heart disease. However, in different studies around the world, the results have been not conclusive. In Mexico, we unknown this polymorphism frequency in patients with ischemic heart disease and controls. **OBJETIVE:** Determination of the CA-13 polymorphism of the endothelial nitric oxide synthase gene frequency in ischemic heart disease patients. **MATERIAL AND METHODS:** We studied 108 patients with ischemic heart disease of the Mexican population and 49 controls. For the entire participant the lipid profile was quantified. For the endothelial nitric oxide synthase gene VNTR polymorphism analysis, we amplified a 130pb segment of intron 13 of the eNOS gene, which consists of 2 pb (CA) repetitions, with 23 different alleles identified. **RESULTS AND CONCLUSION.** The 43 allele was more frequently in patients (32%) vs. controls (10.3%), immediately the 45 allele (30% in patients vs. 1% in controls), however the sample number is not similar and we just conclude the association of this alleles with the ischemic heart disease, this association should be confirm with a wide sample.

Candidate loci linkage analysis in dominantly inherited cataract with microcornea. *C.E. Willoughby^{1,2,3}, A.E. Shafiq⁶, S.B. Kaye⁴, G. Billingsley¹, A. Chandna⁵, E. Heon^{1,2}.* 1) Dept of Ophthalmology/Visual Science Research Program, Toronto Western Hospital Reserach Institute, Toronto, ON, Canada; 2) Dept of Ophthalmology, Hospital for Sick Children, Toronto, ON, Canada; 3) Dept of Medicine, University of Liverpool, Liverpool, UK; 4) Royal Liverpool University Hospital, Liverpool, UK; 5) Royal Liverpool Children's Hospital, Liverpool, UK; 6) Royal Victoria Infirmary, Newcastle-upon-Tyne, UK.

The genetic basis of dominantly inherited cataract with microcornea has not been fully elucidated and is a good model for eye development. To date, the molecular characterization has been limited to one family in whom a mutation in alpha-A-crystallin (CRYAA) was described. The aim of this study is to document the ocular phenotype, and map a genetic locus for dominantly inherited cataract with microcornea, using a three generation family. Seventeen family members (11 affected and 6 unaffected) had a full ocular assessment to document the phenotype. The affected status (presence of cataracts) was determined before the genetic analysis. A panel of 10 candidate loci was selected for preliminary linkage analysis. Microsatellite markers were selected for each locus and genotyping was performed on a Pharmacia automated sequencer. Two-point linkage analysis used the MLINK program of the LINKAGE package v5.2. All affected individuals had congenital cataracts requiring surgery in infancy and 10 individuals also had microcornea. The cataract morphology of one individual was assessed at birth. Linkage analysis of 10 candidate loci (CRYAA, CRYAB, CRYBB2, PAX6, PITX2, PITX3, FOXC1, CHX10, MAF, Nance-Horan syndrome) is reported and provides evidence for another candidate loci in this family. The analysis of this family further outlines the genetic heterogeneity of anterior segment malformation disorders.

Mitochondrial Myopathies in the South African population: implications for diagnosis - lessons from the first four years. A. Olckers^{1,2}, D. Prosser¹, F. Maree^{1,2}, M. Brown³, D. Wallace³, I. Smuts⁴. 1) Centre for Genome Research, Potchefstroom University for CHE, Pretoria, South Africa; 2) DNAbiotec, Pretoria, South Africa; 3) Emory University, USA; 4) University of Pretoria, South Africa.

A Mitochondrial Myopathy (MM) research programme was initiated to investigate the genetic basis of MM in the South African population. This study represents the first extensive investigation of MM in our population and focuses on the most frequent MMs presenting in childhood: MERRF, MELAS and LS. A clinically well characterised group of 53 patients was included: 10 patients indicative of LS, 12 for MELAS and 3 for MERRF. The remaining patients were classified as displaying various non-specific symptoms associated with MMs. Initially our investigative approach included only patients that have been well characterised on the clinical and biochemical level. This remains our approach today, however our molecular investigative strategy has changed dramatically. Initially we screened for the 10 most common MM mutations (3 MERRF, 6 MELAS, 2 LS) in the mt genome via RFLP. After finding that only 1 patient harboured a reported mutation we embarked on an extensive sequencing strategy. Currently our molecular approach is to sequence the 3000, 8000 and 9000 regions. This strategy allows the detection of 13 additional MM mutations. In addition evidence is gathered on the polymorphisms in the region, some of which are African specific, which makes it highly relevant in our population. Failing detection of any causative changes in these 3 regions, we include the individual in our full mt DNA sequencing screening study. The screening of 6 patients has been completed on this level and several known and novel changes have been reported. LS referrals are also subjected to additional analysis of the SURF1 gene via SSCP/HEX. To date 4 patients referred for LS have been identified with aberrant conformers, via SSCP, in exons 3,4,8 and 9 of the SURF1 gene. Diagnosis of MM in the South African population has been challenging, mainly due to the fact that the reported mutations causing MMs in the European population are for the most part not responsible for the MM phenotypes observed in our Caucasian or African populations.

Isolation and characterization of the putative nuclear modifier gene MTO1 involved in the pathogenesis of deafness-associated mitochondrial 12S rRNA A1555G mutation. *M.X. guan*¹, *X.M. LI*², *R.H. Li*³. 1) Div Human Genetics, Children's Hosp Medical Ctr, Cincinnati, OH; 2) same as 1; 3) same as 2.

The human mitochondrial 12S rRNA A1555G mutation has been found to be associated with aminoglycoside-induced and non-syndromic deafness. However, putative nuclear modifier gene(s) has been proposed to regulate the phenotypic expression of this mutation. In yeast, the mutant alleles of MTO1, encoding a mitochondrial protein, manifest respiratory-deficient phenotype only when coupled with the mitochondrial 15S rRNA PR454 mutation corresponding to human A1555G mutation. This suggests that the MTO1-like modifier gene may influence the phenotypic expression of human A1555G mutation. Here we report the identification of full-length cDNA and elucidation of genomic organization of the human MTO1 homolog. Human Mto1 is an evolutionarily conserved protein that implicates a role in the mitochondrial tRNA modification. Functional conservation of this protein is supported by the observation that isolated human MTO1 cDNA can complement the respiratory deficient phenotype of yeast *mto1* cells carrying PR454 mutation. MTO1 is ubiquitously expressed in various tissues, but with a markedly elevated expression in tissues of high metabolic rates including cochlea. These observations suggest that human MTO1 is a structural and functional homolog of yeast MTO1. Thus, it may play an important role in the pathogenesis of deafness-associated A1555G mutation in 12S rRNA gene or mutations in tRNA genes.

(GJB2)Connexin 26 and 30(GJB6) mutations in a South African population with nonsyndromic deafness of probable autosomal recessive origin. *G. De Jong*¹, *C. Whitehead*², *L. Muller*³, *L. Du Plessis*¹, *M.J. Kotze*¹, *L. Warnich*². 1) Dept Human Genetics, Univ Stellenbosch, Tygerberg, South Africa; 2) Dept Genetics, Univ Stellenbosch, South Africa; 3) Department Audiology, Speech and Language, Univ Stellenbosch.

GJB2 And GJB6 gene mutations are common in patients with nonsyndromic autosomal recessive deafness. The most common mutation in the Mediterranean European population with hereditary nonsyndromic deafness is the 35del G mutation in GJB2. Mutations differ in frequency in different countries. A 342kb deletion found within GJB6 has recently been described as a very common in the Spanish population in nonsyndromic autosomal recessive deafness. A second deletion of 140kb was identified in an Ashkenazi Jewish population. In this study we are screening parents with normal hearing and 1 or 2 children with unexplained deafness possibly autosomal recessive ie the deaf children and parents with normal siblings (if available)- at least 50 families. Consent is obtained together with a history as well as blood for DNA analysis. A basic PCR technique was used together with sequencing to detect known and novel mutations. The 35 delG mutation in GJB2 was found at a frequency of ~22% whereas a second mutation, 312del(14) was seen at a frequency of ~16%. In the case of GJB6 the deletion of 140kb has been observed at a frequency of ~21%. The 342kb deletion has not been observed. Further analysis is ongoing.

Large deletion of GJB6 gene in deaf patients heterozygous for GJB2 gene : genotype and phenotype analysis. *S.*

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Hearing loss is the most frequent sensorial defect. At birth, 1/1000 child presents with a severe or profound hearing loss. 60% of the prelingual hearing loss are presumed to have a genetic origin. GJB2 gene represents the major genetic form of prelingual deafness as it accounts for 40% of the congenital hearing loss. GJB2 gene has been analysed in 255 independant patients with non syndromic prelingual hearing loss. 81 of them have a biallelic mutation in GJB2 (35delG 76% ,L90P 4.1%, 167delT2.6%, 312del14, E47X,Q57X 2%) of all mutated alleles. Of the 29 deaf patients heterozygous for a mutation in GJB2 ,16 carry a deletion in trans implicating GJB6 (14 patients 35delG/+, one V37I/+, one Q57X/+). In all families, the molecular anomalies in GJB2 and GJB6 segregate with the hearing impairment. No difference of genotypes between sporadic and familial cases is observed. The phenotype of the 16 composite GJB2/GJB6 heterozygous patients have been compared with the phenotype of patients homozygous for GJB2 mutations or without GJB2 mutations. In profound deafness, the percentage of patients heterozygous composite GJB2/GJB6 and homozygous for GJB2 mutations was significantly higher than in moderate or mild deafness.

A human mitochondrial GTP binding protein related to tRNA modification has an implication for modulating the phenotypic expression of the deafness-associated mitochondrial 12S rRNA mutation. X. Li, M.X. Guan. Div Human Genetics, Children's Hosp Medical Ctr, Cincinnati, OH.

A variety of mitochondrial DNA (mtDNA) mutations have been found to be associated with many clinical abnormalities, including neuromuscular disorders, hearing loss, and diabetes. However, the nuclear backgrounds modulate the phenotypic expression of pathogenic mtDNA mutations, specifically in the deafness-associated A1555G mutation. In yeast, mutant alleles of *MSS1*, encoding a mitochondrial GTP binding protein, manifest a respiratory-deficient phenotype only when coupled with mitochondrial 15S rRNA PR454 mutation corresponding to the human A1555G mutation. This suggests that *MSS1*-like modifier gene may influence the phenotypic expression of the human A1555G mutation. Here we report the identification and characterization of the human *MSS1* homolog, *GTPBP3*. This is the first identified vertebrate gene related to mitochondrial tRNA modification, which plays a pivotal role in the fidelity of translational process. The *Gtpbp3* is the mitochondrial GTPase evolutionarily conserved from bacteria to mammal. Functional conservation of this protein is supported by the observation that isolated human *GTPBP3* cDNA can complement the respiratory deficient phenotype of yeast *mss1* cells carrying the mitochondrial PR454 mutation. *GTPBP3* is ubiquitously expressed in various tissues as multiple transcripts, but with a markedly elevated expression in tissues of high metabolic rates. We also showed that *Gtpbp3* localizes in the mitochondrion by immuno-staining with the specific antibodies and analyzing with a confocal microscope system. These observations strongly suggest that the human *GTPBP3* is a structural and functional homolog of the yeast *MSS1*. Thus, it may modulate the phenotypic manifestation of mitochondrial A1555G mutation.

Molecular and clinical characterization of a large Chinese pedigree with maternally inherited hearing loss. *R. Li¹, X. Bu², M. Yan², M.X. Guan¹*. 1) Dept Human Genetics, Cincinnati Children's Hosp, Cincinnati, OH; 2) Department of Otolaryngology, People's Hospital of Jiangsu Province, Nanjing Medical University, Nanjing, Jiangsu, China 210097.

Mutations in the mitochondrial DNA has been shown to be one of the important causes of non-syndromic sensorineural hearing loss. We reported here the molecular and audiological characterization of a large Chinese family (507 members in six generations) with maternally inherited non-syndromic hearing loss. In particular, 41 subjects in the kernel pedigree in the family were intensively evaluated by audiological approaches and screening for mutations in mtDNA. Audiological results showed symmetrical bilateral progressive sensorineural hearing loss in 17 of 41 individuals with a highly variable age of onset. Sequence analysis of complete mitochondrial genome in this pedigree revealed the presence of a homoplasmic A1555G mutation and other sixteen-nucleotide changes. These are the A663G, and 959 insC in the 12S rRNA gene, five polymorphisms in protein encoding genes, including the A4824G in the ND2 gene, C8794T and A8860G in the A6 gene, A15426G in the cytob gene, and 11 polymorphisms in D-loop region. Of these changes, the novel 959 insC is of special important as the homologous position is the very conserved tRNA and streptomycin-binding region of P-site in the bacterial 16S rRNA. In addition, the 515 CA deletion is the novel mutation in the mitochondrial genome. These data suggests that the A1555G mutation is the primary factor for the deafness phenotype, while other mtDNA mutations, specifically in the 959 insC, acting as the secondary mutation(s), play a synergic role in the deafness phenotype. In addition, the genetic evidence of this pedigree clearly points out that the nuclear modifier gene(s) modulates the phenotypic variability associated with the A1555G mutation. This pedigree is certainly an excellent system for investigating the role of nuclear modifier gene(s) in the development of deafness phenotype related to the A1555G mutation.

Gene expression alterations in tau-deficient mice. *F. Oyama*^{1,2}, *S.E. Kotliarova*², *A. Harada*^{3,4}, *M. Ito*⁵, *Y. Ueyama*^{5,6}, *N. Hirokawa*⁴, *N. Nukina*², *Y. Ihara*¹. 1) Department of Neuropathology, Graduate School of Medicine, the University of Tokyo, Tokyo, Japan; 2) Laboratory for CAG Repeat Diseases, RIKEN Brain Science Institute, Saitama, Japan; 3) Laboratory of Cellular and Molecular Morphology, Institute for Molecular and Cellular Regulation, Gunma University, Gunma, Japan; 4) Department of Cell Biology and Anatomy, Graduate School of Medicine, the University of Tokyo, Tokyo, Japan; 5) Central Institute for Experimental Animals, Kanagawa, Japan; 6) Department of Pathology, School of Medicine, Tokai University, Kanagawa, Japan.

Tau, a microtubule-associated phosphoprotein, plays an important role in determining and maintaining neuronal morphology. Its hyperphosphorylated form is the major constituent of paired helical filaments in Alzheimers disease. A number of exonic and intronic mutations in the tau gene were identified in the patients affected by frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17). The affected brain is characterized by extensive neuronal loss most dominantly in the frontotemporal region and PHF-like fibril formation. Thus, this is definite genetic evidence that tau can kill the neuron. To find an as yet unidentified role of tau in vivo, we used oligonucleotide DNA arrays profiling ~11000 mRNAs to see how the gene expression is altered in the brain of tau-deficient mice, as compared with control mice at the same ages. This microarray screening suggested that the expression of Gem GTPase, a member of Ras-related GTP-binding proteins, is significantly increased at 8 week aged tau-deficient mice. This was confirmed by quantitative RT-PCR. These results indicate that tau-deficiency leads to overexpression of Gem GTPase at 8 weeks of age.

A Behavioral and Correlated Neurochemical Mouse Phenotype Related to the Absence of *Snrpn*, a Locus Associated with Prader-Willi Syndrome. R.A. White¹, E. Vorontosova², R. Chen², S.M. Lunte², M.I. Davies², K.E. Heppert², S.G. McNulty¹, K.N. Young¹, M.G. Butler¹, C.I. Brannan³, T. Thompson⁴, S.C. Fowler². 1) Children's Mercy Hospital and UMKC School of Medicine, Kansas City, MO; 2) University of Kansas, Lawrence, KS; 3) University of Florida, Gainesville, FL; 4) University of Kansas Medical Center, Kansas City, KS.

The bicistronic *Snrpn* gene is involved in mRNA production for two different proteins (SNURF and SmN), and localized in the area that regulates imprinting in the Prader-Willi syndrome chromosomal 15q11-q13 region. Adult homozygous (-/-) and paternally derived (+/-) *Snrpn* knockout mice and adult littermate controls were subjected to three behavioral assays (rotarod, grip strength test, and force-plate actometer). Although the rotarod and grip tests detected no behavioral consequence in the *Snrpn*-deficient mice, the actometer indicated a significant tendency ($p=0.009$) for the *Snrpn*-null mice ($n=6$) to rotate to the right compared to no rotational bias in the controls ($n=12$). The rotational bias suggested possible imbalances in striatal dopamine (DA) levels. Accordingly, the striata of individual mouse brains were dissected into right and left halves and analyzed by HPLC with electrochemical detection for DA content and dopamine metabolites, DOPAC and HVA. The *Snrpn*-deficient mice had lower DA in the left than in the right striatum ($p=0.027$), while the left and right DA levels of controls were similar and at the same level as the right side in the *Snrpn*-deficient knockout mice. The DA metabolites, DOPAC and HVA, also displayed similar *Snrpn*-status-by-side interactions, suggesting that the DA imbalances had not been overcome by compensatory increases in turnover of DA. These neurochemical and behavioral data in the *Snrpn*-null mice, when considered in the context of right striatal overactivity in human Obsessive Compulsive Disorder (OCD), raise the possibility that the *Snrpn*-null mice may model aspects of OCD seen in Prader-Willi syndrome. These findings also suggest that the *Snrpn* knockout mouse may be more relevant to developing an understanding of PWS than had previously been suspected.

Identification of Candidate Genes for Parkinson Disease (PD) by the Convergence of Genetic Linkage and Association Data with Gene Expression in the Substantia Nigra. *M.A. Hauser¹, J.W. Walter¹, R.W. Walters¹, M. Maready¹, S. Takeuchi¹, W.P. Segars¹, C.M. Hulette³, D.E. Schmechel², M.L. Bembé¹, J.M. Stajich¹, E.R. Hauser¹, E.R. Martin¹, B.L. Scott², J. Stenger¹, Y.J. Li¹, F. Beato⁴, R. Jensen⁵, C. Scherzer⁴, S. Gullans⁴, J.M. Vance^{1,2}.* 1) Ctr Human Genetics, Duke Univ Med Ctr, Durham, NC; 2) Dept of Med, Duke Univ Med Ctr, Durham, NC; 3) Dept of Pathology, Duke Univ, Durham, NC; 4) Brigham and Women's Hosp, Harvard Med Sch, Boston, MA; 5) Dept of Physics, Wesleyan University.

Parkinsons Disease (PD) is characterized by the loss of dopaminergic neurons in the substantia nigra. We have reported 5 large linkage peaks in 174 multiplex PD families. Concurrently, we are performing gene expression profiling in the substantia nigra of 3 PD patients (aged 79, 83, and 87) and 2 controls (aged 81 and 72). Expression data often presents so many candidates that it is difficult to interpret. Likewise, linkage analysis of complex disorders yields large regions with many candidate genes. Combining our genetic and expression data allows us to identify critical genes and pathways in the disease, specifically: 1) to assist in the prioritization of candidate genes within linkage peaks and 2) to provide high priority candidate genes for testing of genetic association in our large PD data set. Expression profiling was performed with the Affymetrix U133A gene chip, and by serial analysis of gene expression (SAGE), generating over 200,000 SAGE tags. Genes whose expression levels are significantly up or down regulated in affected individuals constitute candidate susceptibility genes. Of special interest, heat shock protein 90kD (HSP90) protein 1 is up 15-fold ($p=0.000015$), HSP70 protein 4 is up 8-fold ($p=0.003$), and HSP70 protein 5 is up 3-fold ($p=0.0007$) in PD cases vs controls. These results are confirmed by microarray analysis. This is the first report in human tissue corroborating the observation that HSP70 can prevent dopaminergic neuron loss in a *Drosophila* model of PD (Auluck et al. *Science* 295:865). All differentially expressed genes mapping to regions of linkage are being analyzed for their role in the etiology of Parkinson disease.

IDENTIFICATION AND GENOMIC STRUCTURE OF A HUMAN DTNBP1 GENE FROM A PUTATIVE SCHIZOPHRENIA SUSCEPTIBILITY LOCUS ON 6P22.3 IN SILICO. *Y. Jiang¹, R.E. Straub¹, P.F. Sullivan¹, C. Harris-Kerr¹, B.T. Webb¹, B. Wormley¹, X. Wang¹, A. Gibberman¹, A.J. Cesare¹, X. Chen¹, F.A. O'Neill², D. Walsh³, K.S. Kendler¹, B. Riley¹ and I.* 1) Dept Psychiatry, Virginia Inst Psych/Behav Gen, Richmond, VA; 2) Department of Psychiatry, Queens University, Belfast, Northern Ireland; 3) The Health Research Board, Dublin, Ireland.

Schizophrenia is a relatively common, chronic and debilitating psychiatric disorder. It is highly heritable but the genetics are complex. Human chromosome 6p22.3 has previously been linked to schizophrenia in several studies. However, genes present in this region have not yet been investigated in detail. Here we report a full-length cDNA and genomic structure of the human dystrobrevin binding protein 1 gene (DTNBP1) identified between markers D6S260 and D6S1676 by an in silico pipeline and PCR from human brain cDNA libraries. Genome annotation revealed that human DTNBP1 spans 140kb and has a putative promoter with features characteristic of housekeeping gene. We verified 10 exons in our transcript by direct sequencing. The corresponding cDNA is 1349 bp. The open reading frame encodes a 351-amino-acid protein that contains several conserved features: a coiled-coil region, a leucine-rich repeat and several protein kinase phosphorylation sites, which may indicate a role in intracellular signal transduction. It is a promising positional candidate gene for schizophrenia given its genomic localization and interaction with the dystrophin-associated protein complex (DPC) in brain. Three simple sequence repeats and 24 SNPs identified by database hunting and sequencing on pooled ISHDSF DNA samples are useful resources for further evaluating the candidate gene status.

***COL1A1* polymorphism (a four-base-pair insertion polymorphism in 3'UTR) in young male Greek army recruits with lumbar disk disease.** *T.A. Bei*¹, *C. Tilkeridis*², *S. Garatziotis*², *E. Kortetas*^{2,3}, *C.A. Stratakis*^{1,2}. 1) Unit on Genetics & Endocrinology, DEB, NICHD, NIH, Bethesda, MD 20892-1862; 2) Greek Army Medical Center, Arta, Greece; 3) Department of Defense, Greek Army Medical Service, Athens, Greece.

Lumbar disk disease (LLD), one of the most frequent conditions for which patients seek medical care, has been associated with various polymorphisms and/or mutations of the COL genes. *COL1A1*, in particular, has been linked to LLD with a number of genetic changes. In the present study we collected DNA from Greek army young recruits (19-38 years old) and their relatives with LLD. These young soldiers, at the time of their presentation to a military medical training site, were diagnosed with early LLD. All patients had radiological confirmation of their disease; a control group was also studied. A four base-pair insertion polymorphism in the 3' untranslated region (3'UTR) of the *COL1A1* was tested: it is highly polymorphic producing a fragment of 430bp (allele A1) or a fragment of 434bp (allele A2) by simple PCR-amplification. In a total of 30 patients, 10 showed heterozygosity for one of the two alleles; 5 (16.7%) were A1A1, whereas only one of the control subjects had this genotype. Overall, there was a tendency for the shorter allele (A1) to be more frequent in patients with LLD ($p=0.096$). In both groups, there was no deviation from the Hardy-Weinberg equilibrium for this polymorphism of the *COL1A1* gene. We conclude that a previously not studied in LLD polymorphism of the *COL1A1* gene shows a tendency of association with this condition in young soldiers and their relatives; it is unclear whether it is this genetic change that is responsible for a functional effect on the COLA1 protein or whether it is in close association with another COLA1 alteration that is directly responsible. Ongoing studies are aimed at clarifying this question in our patients with LLD.

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Molecular Genetic Dissection of Attention Network Efficiency. *J.A. Fossella, J. Fan, T. Sommer, Y. Wu, D. Pfaff, M.I. Posner.* Sackler Institute, Weill Medical College of Cornell University, New York, NY.

Attention can be viewed in terms of neural networks that carry out specific functions of maintaining the alert state, orienting to sensory input, and coping with stimulus and response conflict. The alerting network shows a dependence on right frontal and parietal areas as well as the effects of noradrenaline; the orienting network involves the posterior parietal lobe and is sensitive to acetylcholine, while the executive network involves frontal areas including the anterior cingulate and lateral prefrontal cortex which are sensitive to dopamine. Our genetic analysis seeks to determine the contributions of noradrenergic and dopaminergic genetic variation to the efficiency of alerting and executive networks, respectively. As a first step, the heritability of each network was gauged using the Attention Network Task (ANT) on 26 pairs of MZ twins and 26 pairs of age matched DZ twins. The highest heritability ($h^2 = 0.89$) was observed for the executive network while alerting showed a lesser heritability ($h^2 = 0.18$) and RT's, while highly correlated among MZ twins (0.74) and DZ twins (0.66), showed modest heritability ($h^2 = 0.16$). ANT efficiency scores have been collected for a mixed population of normal subjects ($N = 400$). PCR-RFLP analysis of buccal swab DNA is used to assay sequence variation in genes involved in dopaminergic and noradrenergic signaling. Statistical associations performed using ANOVA as well as control tests for population stratification using unlinked loci are reported. Sponsored by an NRSA postdoctoral fellowship #1 F32 MH64360-01A1 and NARSAD Young Investigator Award to JAF.

Analysis of Reelin in autism. E. BONORA¹, K.S. BEYER², J.A. LAMB¹, S.M. KLAUCK², A. POUSTKA², A.J. BAILEY³, A.P. MONACO¹, *International Molecular Genetic Study of Autism Consortium (IMGSAC)*⁴. 1) WTCHG, University of Oxford, UK; 2) Department of Molecular Genome Analysis, Deutsches Krebsforschungszentrum, Heidelberg, Germany; 3) Centre for Social, Genetic and Developmental Psychiatry and Department of Child and Adolescent Psychiatry, Institute of Psychiatry, London; 4) <http://www.well.ox.ac.uk/~maestrin/iat.html>.

Autism is a complex neurodevelopmental disorder. Genetic studies indicate that chromosome 7q is likely to contain an autism susceptibility locus. We have used a positional candidate gene approach to identify relevant gene(s) and report here the analysis of Reelin (RELN), a gene located under the peak of linkage. Analysis of a previously reported polymorphic triplet repeat at the 5'UTR and of two intragenic single nucleotide polymorphisms using the transmission disequilibrium test provided no evidence for association with autism in IMGSAC families. The results obtained in the German sample for the triplet repeat showed no evidence of association. Screening the coding regions of RELN in 55 autistic individuals led to the identification of ten missense variants. The exons in which the variants were found were screened in all 169 IMGSAC families and 192 controls. In total, twelve missense variants were identified, five of which were found with similar frequency in controls. Seven variants were not found in controls, three of which did not cosegregate in the affected sibs. Four missense variants were found only in the IMGSAC families and did cosegregate with the disorder in the affected sibs. The four exons in which these variants were identified were screened in an independent sample of 146 German families and 197 German controls. A new missense variant was found and three families carrying two of the four previously identified substitutions were identified. It is difficult to assess the significance of these results in term of contribution to autism susceptibility. The frequency of variants identified is too low to explain the relatively strong linkage results on chromosome 7q. The analysis of RELN suggests that it does not play a major role in autism aetiology, although further analysis is warranted in additional affected individuals.

A genome-wide association study of sporadic amyotrophic lateral sclerosis in Japan. *T. Fukushima*¹, *R. Nakano*¹, *K. Kikugawa*², *T. Inuzuka*³, *A. Miyashita*⁴, *R. Kuwano*⁴, *S. Tsuji*¹. 1) Department of Neurology, Brain Research institute Niigata University, Niigata, Niigata, Japan; 2) Department of Neurology, West Niigata Central Hospital, Niigata, Niigata, Japan; 3) Department of Neurology and Geriatrics, Gifu University, Gifu, Gifu, Japan; 4) Research Laboratory for Molecular Genetics, Niigata University, Niigata, Niigata, Japan.

Purpose: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by a selective loss of upper and lower motor neurons. Approximately 10% of cases are familial, and 15-20% of familial cases have mutations of copper-zinc superoxide dismutase gene. The gene causing an autosomal recessive form of juvenile ALS (ALS2) has also recently been identified. On the other hand, genetic backgrounds of sporadic ALS (SALS), which may be a multifactorial disease, still remains unknown. To find susceptibility loci for SALS, we conducted a genome-wide scan using 811 microsatellite markers. Methods: A total of 84 unrelated Japanese patients with SALS and 95 controls were investigated in this study. Informed consent was obtained from all the subjects. A genome-wide set of 811 microsatellite markers were analyzed using ABI PRISM 3100 Genetic Analyzers. An average distance of each marker is 4.6cM (ABI PRISM Linkage Mapping Set HD-5). The genotyping results were analyzed by the Chi-Square test. For the loci showing the low p value ($p < 0.01$), we conducted detailed association studies using densely distributed microsatellite loci. Results: Among the 811 markers analyzed, we identified one locus with p value < 0.001 on chromosome 18 ($p = 0.00042$) and 8 loci with $0.001 < p < 0.01$. From the detailed association studies, we identified the loci on chromosomes 2 and X with two neighboring loci that gave $0.001 < p < 0.01$. The present study suggests possible SALS susceptibility loci. To further characterize these loci, we are currently conducting a genome scan using more closely distributed markers.

CITED2 is not a major determinant of human NTDs. V. Capra¹, P. De Marco¹, A. Moroni¹, E. Merello¹, A. Raso¹, S. Dunwoodie², A. Cama¹. 1) Lab Servizio Di Neurochirurgia, Inst Scientifico G Gaslini, Genova, Italy; 2) Developmental Biology Unit, Victor Chang Cardiac Research Institute, St Vincent's Hospital, Darlinghurst, NSW 2010, Australia.

Defects of embryonic neural tube closure (NTDs), anencephaly and Spina Bifida, are among the most common human birth defects. Their causes are at least in part genetic, but the genes contributing to human NTDs have not been identified. Maternal periconceptional folate supplementation can reduce risk of NTDs, even if the mechanisms for such preventive effect are until now unknown. Most genetic understanding of neural tube closure is derived from studies of animal models. Gene knock-out technology and spontaneous mutations in mice have produced a series of mutants exhibiting exencephaly or spina bifida, but they differ from the human NTDs in having mendelian, highly penetrant genetic transmission pattern and in having a syndrome of multiple developmental defects. Among them, the Cited2 mutant mouse represents the first genetic model in which folic acid treatment significantly reduces the exencephalic phenotype and prevents a defect in neural tube closure by a mechanism other than compensation of an intrinsic folate related defect in the embryo. In particular, the Cited2^{-/-} mutants died at late gestation and exhibited heart defects and exencephaly, arising from defective closure of the midbrain and hindbrain. Cited2 is a member of a new conserved gene family that comprises genes that bind transcriptional coactivators. It is expressed during mouse development and in adult tissues. In this study we explored the possible involvement of the homologous gene CITED2 in human NTDs malformations by SSCP analysis of 32 patients with myelomeningocele and Chiari II malformation and 31 individuals affected by Chiari I malformation. No crucial shift could be detected in any of the exons examined. Anyway, a possible role of CITED2 in NTDs etiology cannot be excluded because variations in the promoter region and in the introns have not yet been studied. In conclusion, our data, although preliminary, provide evidence that CITED2 does not play a major role in the etiology of human NTDs.

Specific BACE1 Genotypes Provide Additional Risk for Late-Onset Alzheimer Disease in APOE-e4 Carriers. J.-L. Blouin¹, G. Gold², F.R. Herrmann², A. Michon², R. Mulligan², G. Duriaux Saïl¹, C. Bouras², P. Giannakopoulos², S.E. Antonarakis¹. 1) Medical Genetics, University of Geneva School of Medicine and University Hospitals, Geneva, Switzerland; 2) Departments of Psychiatry and Geriatrics, University Hospitals, Geneva, Switzerland.

Alzheimer's disease (AD) is characterized neuropathologically by neurofibrillary tangles and senile plaques in brain. A key component of plaques is Ab, a 40-42 residue polypeptide derived from Ab-precursor-protein (APP) through cleavage catalyzed by secretases (b,g). b-secretase is the rate-limiting enzyme, which represents an alternate to normal a-secretase cleavage. Sequence variation in genes BACE1 (chromosome 11q23.3) and BACE2 (21q22.3), which encode two closely related proteases that appear to act as b-secretase, may represent a strong genetic risk factor for AD. To address this issue, we analyzed the frequencies of 2 SNPs in BACE1 (V262, dbSNP rs#638405) and BACE2 (chr.21-cSNP #hc21s00169, dbSNP rs#12149) respectively in a community-based sample of 96 individuals with late-onset AD followed in geriatric and psychiatric clinics (mean age = 79.9; SD 9.3 ; 45% men) and 170 controls randomly selected among residents of the same community who underwent extensive mental status testing (Epidemiological study of dementia, mean age = 74.7 ; SD 7.4 ; 48% men). Genotype and allele distribution analysis in both groups did not demonstrate any association between AD and BACE1 or BACE2 (Fisher's exact test) in agreement with the recently published studies (Nowotny et al., 2001, Murphy et al., 2001). However, after stratification for APOE status, an association between a BACE1 SNP in codon V262 (genotype GG) and AD in APOE-e4 carriers was observed (p=0.03). Furthermore, when our results are combined with those of Nowotny et al. (2001), the described association becomes highly significant (p=0.003). These results strongly suggest that specific BACE1 haplotypes further increase AD risk in APOE-e4 carrier, possibly through a linkage disequilibrium with other undetected variants in BACE1 which may have a functional effect. (Authors J.-L.B. and G.G. have equal contribution).

Mutation analysis of genes regulating the hypothalamo-pituitary adrenal (HPA) axis in patients with depressive syndrome. *E.B. Binder*¹, *P. Lichtner*², *M. Uhr*¹, *Th. Bettecken*², *T.M. Strom*², *T. Meitinger*², *S. Modell*¹, *F. Holsboer*¹. 1) Max-Planck Institute of Psychiatry, Munich, Germany; 2) Institute of Human Genetics, GSF National Research Center, Munich-Neuherg, Germany.

A series of clinical and preclinical data indicate that a hyperactivity of the HPA-axis may underlie depressive symptomatology. We have shown that depressed patients can be divided into two groups: one showing a drastical hyperactivity of the HPA-axis that normalizes with remission; the other not displaying an increased activity of the HPA-axis. HPA-axis activity was measured using the Dexamethasone suppression/corticotropin releasing hormone-stimulation (Dex-CRH) test within 10 days of admission to the psychiatric ward. Hamilton Depression Rating Scale scores at the time of the test were above 18 in all patients and did not differ between the groups. These two subgroups may thus represent two biologically and possibly genetically distinct subtypes of depressed patients. To investigate whether these subtypes differ with respect to polymorphisms in genes regulating the HPA-axis, we screened the following seven genes for polymorphisms: CRH and vasopressin (AVP), which regulate the axis at the hypothalamic level, and BAG1, STUB1, P23, FKBP4, FKBP5, which are chaperones of the glucocorticoid receptor that have been shown to regulate glucocorticoid receptor function in *in vitro* experiments. Polymorphism screening was performed using capillary electrophoresis SSCP analysis and subsequent sequencing in 47 depressed patients with hyperactive HPA axis vs. 47 patients with normal HPA-axis activity. Up to 1000 bp of the 5 untranslated region and all exons, including exon/intron junctions were analyzed. Several rare and novel polymorphisms could be identified. Some of these polymorphisms will be tested for association with HPA-axis activity in depressed patients as well as for association with depressive symptomatology in a case/control sample. To that aim genotyping will be performed in 188 patients with Dex-CRH test and 188 healthy age- and sex-matched controls.

PICK1, a gene on chromosome 22q13: a putative regulator of serine racemase and D-serine. *K. Fujii^{1,2}, J. Xia¹, P. Dulloor¹, Y. Ozeki¹, N. Yamada², M. Ohkawa², C.A. Ross¹, S.H. Snyder¹, R.L. Huganir¹, A.E. Pulver¹, A. Sawa¹.* 1) Depts. of Psychiatry and Neuroscience, Johns Hopkins Univ. Sch. of Med. Baltimore, MD; 2) Shiga Univ. of Med.Sci. Otsu, Japan.

There has been growing evidence suggesting that dysfunction or down-regulation of NMDA neurotransmission may contribute to the manifestations of schizophrenia. D-serine is a putative endogenous co-agonist for the glycine site of NMDA receptor, and clinical trials of D-serine with classical neuroleptics can improve part of the symptoms in schizophrenic patients, suggesting a role of D-serine in schizophrenia. We have evaluated roles of serine racemase (SR), an enzyme synthesizing D-serine, in the pathogenesis of schizophrenia. Through yeast two-hybrid screenings, we have identified protein interacting with C kinase (PICK1) as a potential interacting molecule with SR. By yeast two-hybrid assay and in vitro protein binding assay, we confirmed that the PICK1/SR binding requires PDZ domain and coiled-coil domain in PICK1. In cellular models, PICK1 can contribute to re-distribution of SR; this redistribution is specifically augmented by the addition of TPA, a protein C kinase activator. The gene for PICK1 is located on chromosome 22q11-13, a schizophrenia susceptibility locus in linkage analyses. Based on our hypothesis that genetic variations (mutations, polymorphisms) in the PICK1 gene may interfere with SR enzymatic activity, D-serine production, and NMDA neurotransmission in schizophrenia, we have analyzed the PICK1 gene in schizophrenic probands whose families demonstrated excess allele sharing among the affecteds in this region. We have found a couple of novel polymorphisms in PICK1 gene through sequence analyses in the patient samples. The polymorphisms examined thus far are located in intronic regions just adjacent to exon-intron boundaries. We are extending the sequencing analyses to normal populations and sporadic schizophrenics. We are also analyzing whether these novel intronic polymorphisms influence exon usages as well as functions of PICK1 and SR proteins.

Evidence for allelic association between three chromosome 8p21.3-22 markers and schizophrenia points to the brain expressed microtubule associated protein gene, Pericentriolar Material (PCM1) as a candidate for increasing genetic liability to schizophrenia. *H.M.D. Gurling¹, E. Blaveri¹, S. Datta¹, G. Kalsi¹, J. Lawrence¹, D. Quested⁵, H. Moorey³, G. Lamb³, U. Chowdhury³, D.H. Blackwood⁴, W.J Muir⁴, D. Curtis².* 1) Molecular Psychiatry Lab. Windeyer Inst, University College London Med Sch, London, England; 2) City and East London Mental Health Trust, Royal London Hospital, Whitechapel, London, UK; 3) Camden and Islington Mental Health and Social Care Trust, St Pancras Hospital, London, UK; 4) Department of Psychiatry, University of Edinburgh, Royal Edinburgh Hospital, Morningside Park, Scotland, UK; 5) West London Mental Health Trust, St Bernards Hospital, Ealing, London, UK.

Three linkages to a chromosome 8p21-22 schizophrenia locus with lods above 3.00 have been reported (Blouin 1999, Brzustowicz 1999, Gurling 2001). In a case control study of 137 schizophrenics of UK ancestry and 316 matched controls, 3 markers; D8S2615, D8S2616 and D8S261, all within 108 kb, showed allelic association with schizophrenia with p values of 0.004, 0.024 and 0.01, respectively, after Monte Carlo correction for multiple alleles. Replication in a 2nd sample of 100 Scottish cases and 100 controls was found with D8S2616 ($p < 0.04$ after MC correction) but not in the 3rd sample of 100 cases and 100 controls. The combined UK and Scottish cases (N 337) and controls (N 516) showed significant association for all three markers after correction. We have identified a narrow region of 8p22, containing only the PCM1 gene, as being involved in the cause of schizophrenia. We sequenced the PCM1 gene in chromosome 8p22 associated schizophrenic cases and found several exonic base pair variants and insertion/deletions which still need to be investigated fully in relation to schizophrenia. If confirmed, better methods for diagnosis and prognosis can be created. The pinpointing of a new protein target and its neurochemical pathway will lead on to better prevention and new drug treatments for schizophrenia. Blouin JL 1999 *Nature Genetics*, 20, 70-73. Brzustowicz LM 1999 *Am J Hum Genet* 65,1096-1103. Gurling HM 2001 *Am J Hum Genet* 68, 661-673.

Genetic markers showing linkage disequilibrium with a chromosome 8p22 schizophrenia susceptibility locus near the pericentriolar material gene (PCM1) are associated with reduced left orbitofrontal cortex grey matter volume and other cerebral morphology changes at MRI compared to non chromosome 8p22 associated schizophrenics. *E. Blaveri¹, H.D. Critchley², H.M.D Gurling¹, O. O'Daly¹, G. Kalsi¹, S.R. Datta¹, A. McQuillin¹, H. Moorey³, D. Quested⁴, D. Curtis⁵, J. Lawrence¹, N. Bass¹, C.D. Good², R.S. Frackowiak², R. Dolan².* 1) Molecular Psychiatry Lab. University College London, London, England, UK; 2) Functional Imaging Laboratory, Wellcome Department of Imaging Neuroscience, Institute of Neurology, University College London, England, UK; 3) Camden and Islington Mental Health and Social Care Trust, St Pancras Hospital, London, UK; 4) West London Mental Health Trust, St Bernards Hospital, Ealing, London, UK; 5) City and East London Mental Health Trust, Royal London Hospital, Whitechapel, London, UK.

In 137 unrelated UK cases of schizophrenia and 316 matched controls we found three markers showing significant allelic association with schizophrenia at or near the PCM1 gene locus after correction for multiple alleles. PCM1 is a microtubular associated protein and could increase liability to schizophrenia. Fourteen schizophrenic patients who had inherited the chromosome 8 alleles associated with schizophrenia at two out of the three loci volunteered for brain morphology studies using MRI. They were compared to a sample of non chromosome 8 associated schizophrenics and two matched normal control groups. MRI data was analysed with age and gender as confounding covariates and corrected for total intracranial volume. A region of left orbito-frontal cortex showed significantly reduced grey matter in chromosome 8 associated schizophrenics (SZ8) compared to non chromosome 8 associated schizophrenics (SZ0) or controls ($p=0.003$). Differences in combined grey plus white matter and grey matter volumes between SZ8 patients and normal controls reached significance whereas no differences were found for these volumes between SZ0 cases and normal controls. SZ8 cases also demonstrated significant reductions in inferior and anterior thalamic grey matter volume and total brain volume compared to SZ0 cases.

Saturation mapping of bipolar affective disorder (BPAD) susceptibility locus on chromosome 20p11.2-q11.2. A.N. Akarsu¹, S. Kara-Ozer², Z. Yuncu³, B. Girgin¹, H. Okur¹, A. Sir⁴, H. Herken⁵, S. Senol⁶, U. Radhakrishna⁷, J.L Blouin⁷, R. Lyle⁷, S. Vahip³, A. Gogus², A. Ulusahin², S. Antonarakis⁷ and Bipolar Genetics Study Group of Turkey. 1) Gene Mapping Laboratory, Ped. Res. Cent. Hacettepe Univ. Ankara and Psychiatry Departments of; 2) Hacettepe; 3) Ege; 4) Dicle; 5) Gaziantep; 6) Gazi Universities, Turkey; 7) University of Geneva Medical School, Switzerland.

We have previously identified a strong linkage to chromosome 20p11.2-q11.2 in a large autosomal dominant Turkish family with BPAD (*Eur J Hum Genet* 9: 39-44, 2001). We have further initiated a systematic sample collection strategy which targets large families, sib pairs, parent offspring trios as well as sporadic cases and controls to reduce the critical interval. We have also updated the original pedigree (BP-TU1) by adding new members and reexamined younger generation (not affected by the time of the study). We have now over 600 samples from 254 index cases including 90 parent-offspring trios and 26 multiplex families. Suspicious segregation between the disease allele and the DNA markers from 20p region was observed only in 2 out of 26 families. Since we have observed common alleles for the markers D20S880 and D20S481 in these families a case control study has been performed in 158 affected samples and 130 controls (age and ethnically matched). Marker D20S481 gave 11 alleles and allele 5 (237 bp) was the most frequent allele in Turkish population with an allele frequency of 0.335. A significant association between the allele 4 (241 bp) and BPAD phenotype was observed ($p=0.042$). Furthermore, several affected individuals ascertained from the vicinity of BP-TU1 pedigree demonstrated partial haplotype association between the DNA markers D20S911, D20S880, D20S119, D20S481, D20S1151 harbouring D20S119 and D20S481 core. KCNS1 is a member of voltage gated potassium channels and is expressed only in the brain. This gene was also physically mapped to the same region with markers D20S119 and D20S481. Mutation screening in the coding region of KCNS1 gene is in progress. Support: Hacettepe University Research Foundation(Grant:01 01 101 001). nakarsu@hacettepe.edu.tr.

Association between DRD4 VNTR allele-length and the amplitude of event related potentials (ERP) during a visual attention task in attention deficit/hyperactivity disorder (ADHD) children. A. Birca, I. Fortier, L.R. Simard, P. Robaey. Hôpital Sainte-Justine, Montral, Qc, Canada.

Both genomewide and candidate gene screens have been used to identify susceptibility genes of ADHD, a complex trait with a strong genetic component. The best candidates to date are the dopamine transporter (DAT1) and D4 receptor (DRD4) genes but these associations have not always been replicated. There are a number of methodological reasons for this discordance including extensive clinical heterogeneity. Therefore, we hypothesized that more consistent associations could be revealed within ADHD children by using behavioural and brain electrical responses obtained during a visual attention task. To this end, 31 ADHD children from 6 to 9 years of age (22 boys and 9 girls) undertook a selective visual attention task during which EEGs were recorded and blood samples drawn to genotype the 48-bp VNTR in exon 3 of the DRD4 gene. ADHD children were divided into 2 groups: those with long alleles (³⁷R) and those without (<7R). Association between the DRD4 genotype and performance measures (mean reaction time, intra-subject variability and number of correct responses) or the amplitude of two ERP components: P450 (parietal sites) and N530 (anterior frontal sites) over the left and right hemispheres to target and non-target stimuli was assessed by ANOVA. No group differences were found for performance measures. On the other hand, there was a significant main effect of genotype on the P450 amplitude ($F=4.49$, $p=0.043$). This effect was greatest over the right hemisphere in response to non-targets (21.6 ± 9 mV vs. 13.5 ± 11.1 mV in ³⁷R vs. <7R groups, respectively; $t=-2.14$, $p=0.041$). We also observed a positive correlation between VNTR allele length and P450 amplitude ($R^2=0.418$, $p=0.019$). No group differences were observed for N530; however, we did detect a tendency towards higher N530 amplitudes over anterior frontal sites in the ³⁷R group, especially in response to non-target stimuli. This pilot study represents the first step towards linking sequence variants in potential ADHD susceptibility genes and specific phenotypes of brain function. Funded by FRSQ and HSJ.

CYP26A1 and RALDH2 are not implicated in the development of human neural tube defects. *M. Dickerson¹, J. Joseph¹, T.M. George², D.S. Enterline³, E.C. Melvin¹, J.R. Gilbert¹, E. Linney⁴, M.C. Speer¹ and NTD Collaborative Group.* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Department of Surgery, Duke University Medical Center, Durham, NC; 3) Department of Radiology, Duke University Medical Center, Durham, NC; 4) Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC.

Retinoids (vitamin A and its derivatives) are necessary for normal growth and development of the embryo. CYP26A1 and RALDH2, genes whose products are responsible for positioning and limiting the influence of retinoic acid in the developing embryo, have a synergistic relationship. RALDH2 helps metabolize Vitamin A into retinoic acid so it can be used by the cells; CYP26A1 serves to degrade or confine retinoic acid in order to protect the cells from exposure to excess amounts particularly in the caudal end. Mis-regulation of retinoic acid exposure to the developing embryo is responsible for a variety of birth defects, including neural tube defects. This relationship has been identified through studies both in mouse and zebrafish. We investigated these genes using denaturing high performance liquid chromatography (DHPLC) as candidate genes for neural tube defects (NTD)s. The screen included 477 cases with NTD, organized into 95 pools with approximately 5 cases each. Coding sequences for the 13 exons in RALDH2 and 7 exons in CYP26A1 have been evaluated. SNPs have been identified in intron 8 and exon 11 of RALDH2 and, to date, no variation has been identified in CYP26A1. Thus, these results suggest that RALDH2 and CYP26A1 are unlikely to represent major genes that influence the development of neural tube defects in humans.

APOE- e4 in association with persistent neurocognitive impairment after traumatic brain injury. D.B.

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Neuroimaging, neurophysiologic, and neurogenetic studies may improve understanding of the neurobiological bases of impaired cognition due to mild TBI, and may be of service in the development of rationale therapies for persistently impaired TBI survivors. We reported convergent evidence of structural and functional hippocampal abnormalities among persons with chronically impaired sensory gating, attention, and memory following TBI at all levels of initial severity. While we interpreted the comparable neurobiological findings in these groups as a reflection of our subject selection methods (relatively unfavorable outcome following mild TBI and relatively good outcome following severe TBI), the observation of comparable structural and functional abnormalities between groups with disparate initial injury severity requires further explanation. The APOE-e4 genotype has been associated with relatively poor cognitive, motor, and functional outcome following repetitive injury or severe TBI. Our subject selection procedures produced a relatively "poor outcome" mild TBI group and a relatively "good outcome" moderate/severe TBI group; we hypothesized that the frequency of the APOE-e4 genotype would be highest in the former group. APO-e4 genotypes of 26 subjects with mild or moderate/severe TBI participating in our previous studies were determined. 11/26(42%) TBI subjects are carriers of one APOE-e4 allele. Among those with mild TBI, 9/18 (50%) are APOE-e4 carriers, while only 2/8 (25%) with moderate-severe TBI are APOE-e4 carriers. Binomial statistical comparison of the frequency of APOE-e4 status in the mild TBI group differs significantly ($p < .03$) from the APOE-e4 expected frequency (28%) in a demographically comparable normal population. These preliminary results suggest that the APOE-e4 genotype may be an important factor in outcome following mild TBI, and may be useful in the effort to understand the basis of relatively poor cognitive outcome experienced some ostensibly "mildly" injured patients.

Modifier effects in autism at the DBH and MAO-A loci. *M.B. Jones¹, R. Palmour², L. Zwaigenbaum³, P. Szatmari⁴.*
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Autism is a neuropsychiatric disorder that presents with marked variation in symptoms and adaptive functioning. The disorder is caused in large part by genetic mechanisms though no disease genes have yet been identified. The objective of this study was to investigate the role of the DBH and MAO-A genes in increasing risk for the disorder and in modifying the severity of the phenotype, specifically IQ. We assembled 67 sib pairs and 45 singletons with an autism spectrum disorder as determined by the ADI-R, ADOS and best-estimate diagnosis. Two different markers were genotyped in the DBH gene and one in the MAO-A gene. Allele frequencies among affected children and first degree relatives were determined and susceptibility effects through both the maternal and fetal genotypes were estimated using log linear models. Modifier effects were assessed by comparing the number of autistic symptoms and IQ of the affected children as a function of the maternal and fetal genotypes. Sizeable and significant modifier effects on IQ in affected children were found at both the DBH and MAO-A loci. For example, the low activity alleles at the DBH locus were associated with higher IQ among affected children. No modifier effects were found when autistic symptoms were compared as a function of maternal or fetal genotype. Susceptibility effects were also found but fewer, weaker and more open to question. DBH appears to act both in the mother (via the intra-uterine environment) and in the fetus to modify level of functioning whereas MAO-A acts only in the mother. The results are consistent with the hypothesis that in children at risk for autism, high levels of dopamine in utero are associated with higher IQ and low levels with lower IQ. The results have implications for the role of dopamine in autism and for the detection of modifier genes in other complex genetic disorders.

***PRODH* mutations and hyperprolinemia in schizophrenic patients.** H. Jacquet¹, G. Raux¹, F. Thibaut^{1, 2}, B. Hecketsweiler³, E. Houy^{1, 2}, C. Demilly², S. Haouzir², G. Allio², V. Drouin⁴, J. Bou¹, M. Petit^{1, 2}, D. Champion^{1, 2}, T. Frebourg^{1, 4}. 1) INSERM EMI 9906, Faculty of Medicine, Rouen; 2) Department of Psychiatry; 3) Department of Biochemistry; 4) Department of Genetics, CHU de Rouen, 76031 Rouen, France.

The increased prevalence of schizophrenia among patients with the 22q11 interstitial deletion associated with the DiGeorge syndrome has suggested the existence of a susceptibility gene for schizophrenia within the DiGeorge syndrome chromosomal region (DGCR) on 22q11. Screening for genomic rearrangements of 23 genes within or at the boundaries of the DGCR in 63 unrelated schizophrenic patients and 68 unaffected controls, using Quantitative Multiplex PCR of Short Fluorescent Fragments (QMPSF), led us to identify, in a family including two schizophrenic subjects, an heterozygous deletion of the entire *PRODH* gene encoding the proline deshydrogenase. This deletion was associated with hyperprolinemia in the schizophrenic patients. In addition, two heterozygous *PRODH* missense mutations (L441P and L289M), detected in 3/63 schizophrenic patients but in none among 68 controls, were also associated with increased plasma proline levels. Segregation analysis within the two families harboring respectively the *PRODH* deletion and the L441P mutation showed that the presence of a second *PRODH* nucleotide variation resulted into higher levels of prolinemia. In a patient suffering from type I hyperprolinemia with severe neurological manifestations, we identified an homozygous L441P and an heterozygous R453C mutation of the *PRODH* gene. These observations suggest that the genetic determinism of type I hyperprolinemia, characterized by a deficiency of proline deshydrogenase enzyme, is complex, the severity of hyperprolinemia depending on the number of hits affecting the *PRODH* locus and demonstrate that type I hyperprolinemia is present in a subset of schizophrenic patients.

Allelic variation at genes encoding NRCAM and NrCAM ligands and possible associations with substance abuse vulnerability. *H. Ishiguro, G. Uhl.* Molecular Neurobiology, NIDA, NIH, Baltimore, MD.

NrCAM is a single-TM domain protein that binds to ligands expressed on other cells in ways that alter cell contacts and are likely to change tyrosine kinase activities. In initial association-based scans for drug abuse vulnerability alleles in a chromosome seven region linked to alcoholism by COGA investigators, we have identified an NrCAM haplotype that includes 3 silent or missense SNPs which is present at lower frequencies in several samples of Caucasian substance abusers and higher frequency in a sample of African-American polysubstance abusers. We have separately identified NrCAM as a morphine- regulated gene in subtracted hybridization/PCR differential display studies, and found that it is expressed by neurons in brain regions that could play important roles in memory and addiction. To help elucidate possible roles for NrCAM locus variation in addiction vulnerability, we have examined association of markers at the loci of the genes for NrCAM ligands expressed in brain including: CHL1, contactins 2(axonin/TAG-1) and 5, receptor protein tyrosine phosphatase beta (RPTPbeta)and RPTPbeta homolog. We have compared marker frequencies in 177 unrelated Caucasian alcoholics and controls from the COGA study. Markers that displayed nominal significant differences were tested using Caucasian and African American abuser and control individuals recruited at the NIDA-IRP. An RPTPbeta gene haplotype was defined by SNP variants that were 1.3-1.4-fold more abundant in abusers. Abusers who displayed both the Pro526 NrCAM and 127G RPTPbeta alleles were 1.5-3 fold more frequent than controls. These data provide additional evidence that now tentatively implicate several NrCAM gene family members in substance abuse vulnerability.

Search for genetic contribution to the neurotoxic effects of thimerosal (ethylmercury) in mouse strains. *D.Y. Kim, J. Gitschier.* HHMI, University of California, San Francisco, CA.

Thimerosal, a commonly used preservative that is 49.6% ethylmercury by weight, has been implicated as a potential cause of autism. We hypothesize that genetic susceptibility to mercury toxicity could underlie the development of autism in some cases. To assess genetic influences in an animal model, we have begun to investigate the effects of thimerosal on the inbred mouse strains C57BL/6, FVB/N, BALB/cBy, 129S1/SvIm, and the outbred strain CD1. Mice from these different strains have been given a one-time subcutaneous injection between postnatal days 7 to 16. They have been observed and followed weekly using the SHIRPA protocol, an assessment used for general behavioral and functional testing. Preliminary results have shown that death typically occurs at a dose of 100mg/kg. At 50mg/kg, the C57BL/6 strain test group demonstrates hyperarousal and delayed eye opening compared a buffer-injected C57BL/6 group at approximately 2 weeks post-injection. However, by post-injection week 5, the experimental and control groups appear to perform comparably upon testing. In addition, within the CD1 outbred strain we may have identified several mice that display unusual resistance to thimerosal's effects. One CD1 mouse out of 16 survived a 300mg/kg dose of thimerosal and 5 out of 15 mice have survived a dose of 200mg/kg. On SHIRPA testing, the survivors perform as well as control animals even at 70 days post-injection, which suggests that these mice may represent a thimerosal-resistant strain. We are in the process of breeding these mice and will be observing the progeny for their response to thimerosal. Also, we are testing other inbred strains at a dose of 75mg/kg, a sublethal dose that is likely to elicit some neurologic effect based on our preliminary data.

DISC-1: Its function and possible implication for mental illnesses. *Y. Ozeki¹, K. Fujii^{1,2}, J. Kleiderlein¹, U. Park¹, X. Luo¹, A. Kamiya², N. Yamada², M. Ohkawa², S.H. Snyder¹, C.A. Ross¹, A. Sawa¹.* 1) Department of Psychiatry and Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Department of Psychiatry, Shiga University of Medical Science, Otsu, Shiga, Japan.

DISC-1 (Disrupted-in-Schizophrenia-1) was identified as a transcript with an open reading frame that is disrupted as a result of translocation in a large Scottish family with major mental illness including schizophrenia and mood disorders. There has been growing evidence that DISC-1 may be a candidate gene for such psychiatric illnesses. We have investigated expression of DISC-1 protein in the brain using several antibodies we have raised. DISC-1 protein expression was more prominent in developing brains when compared with adult brains. In cellular models, DISC-1 protein was induced in PC12 cells upon their differentiation by NGF. To clarify physiological roles of DISC-1, we have performed the yeast two-hybrid screen. Most of the DISC-1 interactors have roles in neural development. We have already confirmed the interaction and co-distribution of NUDEL and DISC-1 with biochemical and immunofluorescent methods. To analyze DISC-1 functions further, we have also tried overexpression or knocking-down expression of DISC-1 in cellular models. We have found that DISC-1 self-dimerizes (or oligomerizes) in cells, which was addressed by co-immunoprecipitation experiments. Such self-dimerization occurred not only between full-length DISC-1 proteins, but also between the C-terminal truncated DISC-1 proteins (the putative disease protein resulting from the chromosomal translocation and the subsequent DISC-1 gene disruption) as well as the combination of full-length and C-terminal truncated DISC-1. These results are consistent with gain of function or dominant negative models in dominant genetic transmission, although we do not exclude the possibility of a haploinsufficiency model.

Polyglutamine Binding Peptide 1 (QBP1) inhibits polyglutamine aggregation and rescues neurological phenotypes in *Drosophila* polyglutamine disease models. Y. Nagai¹, N. Fujikake², K. Ohno⁴, H. Higashiyama², T. Inui³, H.A. Popiel¹, Y. Urade³, M. Yamaguchi⁴, W.J. Strittmatter⁵, J.R. Burke⁵, T. Toda¹. 1) Div Functional Genomics, Dept Post-Genomics&Diseases, Osaka University Graduate School of Medicine, Suita, Japan; 2) The Fourth Dept; 3) The Second Dept, Osaka Bioscience Institute, Suita, Japan; 4) Chr Tech Group, Div Biotechnology, Kyoto Institute of Technology, Kyoto, Japan; 5) Dept Medicine (Neurology), Duke University Medical Center, Durham, NC.

Polyglutamine (polyQ) diseases are a growing class of at least nine inherited neurodegenerative diseases including Huntingtons disease, various types of spinocerebellar ataxia, and spinobulbar muscular atrophy. They are caused by abnormal expansions of the polyQ stretch in each unrelated disease protein. Expansion of the polyQ stretch is considered to confer toxic properties on the disease proteins through alterations in their conformation, leading to pathogenic protein-protein interactions including aggregate formation. By phage display screening, we previously identified Polyglutamine Binding Peptide 1 (QBP1), a peptide sequence that preferentially binds the expanded polyQ stretch and is expected to interfere with the pathogenic properties of the expanded polyQ stretch. We showed the ability of QBP1 to inhibit polyQ protein aggregation *in vitro* and to suppress polyQ-induced cell death in cell culture. Using *Drosophila* polyQ disease models, we show here that QBP1 inhibits polyQ aggregation, suppresses polyQ-induced compound eye degeneration, and most importantly rescues polyQ-induced premature death. Structural analysis using circular dichroism suggests that QBP1 inhibits aggregation of the expanded polyQ protein by masking its binding surface rather than by affecting its conformation. Selective inhibition of the pathogenic interactions of expanded polyQ stretches by QBP1 is a useful strategy for developing potential therapies for the currently untreatable polyQ diseases.

The 5HT1Db Receptor Gene in Autism: Case-Control Study and Genotype-Phenotype Correlation Analysis in Multiplex Families. *X. Liu¹, E. Mundo², C. Zhang¹, JJA. Holden¹.* 1) Department of Psychiatry & Physiology, Queen's University, Cytogenetics & DNA Research Lab, Ongwanada Resource Centre, Kingston, ON, Canada; 2) Centre for Addiction and Mental Health, Clarke Site, University of Toronto, ON, Toronto, Canada.

Autism is a severe neurodevelopmental disorder with a complex genetic etiology, likely involving multiple genes. Increased blood and urinary serotonin (5HT) levels, the positive effects of 5HT re-uptake inhibitors on symptoms in autistic patients, and the proven role of 5HT in neurodevelopment have prompted extensive investigations of the serotonin system genes in autism. 5HT1Db gene has earned increased attention because of its essential role in the regulation of 5HT release and the use of 5-HT1Db receptor agonists to ameliorate autism-related symptoms in other psychiatric disorders. We therefore genotyped the G861C SNP in the 5-HT1Db gene in two sets of multiplex autistic families: 47 Canadian families and 81 US families (the latter were from AGRE). Case-Control comparison showed: Allele C, genotype GC and genotype CC have significantly higher frequencies in affected children from Canadian families compared to Canadian controls ($\chi^2 = 5.081$, $p = 0.024$, $df = 1$ for allele; $\chi^2 = 6.890$, $p = 0.032$, $df = 2$ for genotype; if GC and CC combined, $\chi^2 = 5.941$, $p = 0.015$, $df = 1$). A similar, non-significant trend was seen in the AGRE families. Genotype-phenotype correlation analysis using ADI-R data, showed that children with one or two copies of the C allele have significantly more severe impairment in communication ($F = 10.546$, $p = 0.002$) and social interaction ($F = 4.266$, $p = 0.04$) compared to children without the C allele. Strikingly, almost all (98%) autistic children with one copy of the C-allele had severe communication problems compared to only 80% of those homozygous for the G-allele ($\chi^2 = 7.411$, $p = 0.006$, $df = 1$). Taken together, either the G861C polymorphism in the 5HT1Db gene or a closely linked variation in 5HT1Db or another adjacent gene, appears to be a risk factor that modifies symptoms in persons with autism. (Supported by grants to JJAH from the CIHR and the OMHF).

APOE Promoter Region SNPs, APOE Genotype, and risk of Alzheimer Disease (AD) in Cases, Controls, and Families. *K.K. Nicodemus¹, C. Allen¹, A.D. Roses², A.M. Saunders¹, G. Small³, D. Schmechel¹, P.M. Conneally⁴, J.L. Haines⁵, J.G. Gilbert¹, J.M. Vance¹, M.A. Pericak-Vance¹.* 1) Ctr Human Genetics, Duke Univ Med Ctr, Durham, NC; 2) GlaxoSmithKline, Research Triangle Park, NC; 3) Univ of CA at LA; 4) IN Univ, Indianapolis IN; 5) Prgrm Human Genetics, Vanderbilt Univ Med Ctr, Nashville TN.

APOE is the only identified genetic risk factor for late-onset AD, although the presence of the APOE-4 allele is neither necessary nor sufficient. It may be that SNPs in the promoter region (PR) of AD modulate transcription of APOE4. Although several have considered individual SNPs (A491T, C427T, G219T, and C113G) and 2-SNP haplotypes in case/control studies, ours is the first to assess the risk associated with these SNPs and combinations of these SNPs in families as well as in cases/controls. We also typed an additional SNP (C5361T) with an unknown effect on risk of AD. Because the PR SNPs are physically close to the SNPs that are used to determine APOE genotype, we expected strong linkage disequilibrium between the PR SNPs and APOE genotype, and thus all analyses were stratified by APOE genotype. In a sample of 45 discordant sibship families where all individuals were APOE 3/4, association results with individual SNPs showed increased risk with 491A ($p=0.02$) and 427T ($p=0.03$). Global tests for 2-SNP haplotypes revealed a positive association between 491A-427T haplotype and AD (single haplotype $p=0.008$; global $p=0.06$). However, in families where all individuals were APOE 4 negative ($n=26$), single SNP and 2-SNP haplotype analysis revealed no association between APOE PR SNPs or haplotypes and risk of AD. Confirming a previous study, in APOE 3/3 cases versus controls ($n=378$) we observed an increase in risk of AD in individuals who carried the 113G allele (OR=1.77; 95% CI 1.07, 2.92; $p=0.03$), and we also found an increased risk associated with the 113G-5361T haplotype in this group (haplotype $p=0.004$; global $p=0.04$). In conclusion, we found evidence that APOE PR SNPs may play a small role in increasing the risk of AD in individuals who have one or no APOE-4 alleles.

Association between catechol-O-methyl transferase gene polymorphism and wearing-off and dyskinesia in Parkinson's disease. *M. Watanabe*¹, *S. Harada*², *T. Nakamura*², *N. Ohkoshi*¹, *K. Yoshizawa*³, *A. Hayashi*¹, *S. Shoji*¹. 1) Neurology/Inst Clinical Sci, Tsukuba Univ, Tsukuba, Japan; 2) Legal Medicine/Inst Community med, Tsukuba Univ, Tsukuba, Japan; 3) Neurology National Mito Hospital, Mito, Japan.

Catechol-O-methyl transferase (COMT) is an enzyme that inactivate catecholamines, including levodopa. An amino acid change (Val-108-Met) in the COMT protein has been found to result in a change from high to low enzyme activity. In the present study we genotyped 121 Japanese patients with Parkinson's disease (PD) and 100 controls. Comparison of the allele frequencies revealed that homozygosity for the low-activity allele was significantly more common among PD patients than the control ($P=0.047$, odds ratio=3.23). In addition, homozygosity for the low-activity allele was over-represented in PD patients that exhibited the "wearing-off" phenomenon ($P=0.045$, odds ratio=3.82) or dyskinesia ($P=0.030$, odds ratio=4.80) compared to controls, although these differences were not significant after Bonferroni's correction. Our results may help in the understanding of the mechanism that cause complication of levodopa therapy in PD patients.

High resolution haplotype analysis of the PSEN1 gene. *H. Yamagata*^{1, 2}, *M. Matsubara-Tsutsui*², *K. Kamino*³, *J. Nakura*², *A. Morishima*⁴, *N. Mitsuda*⁴, *I. Kondo*¹, *T. Miki*². 1) Dept Hygiene, Ehime Univ Sch Medicine, Ehime, Japan; 2) Dept Geriatric Medicine, Ehime Univ, Ehime, Japan; 3) Dept Clin Neuroscience, Osaka Univ, Osaka, Japan; 4) 2nd Dept Human Physiology, Ehime Univ, Ehime, Japan.

We sought to establish an association between sporadic Alzheimer disease (AD) and presenilin 1 (PSEN1) gene polymorphisms in the Japanese population. A total of 189 AD cases (NINCDS-ADRDA criteria) and 240 controls were studied. A 5kb fragment containing the putative promoter of the PSEN1 gene for randomly selected control subjects was subcloned into plasmid and sequenced to screen novel polymorphisms on this region. Patients and controls were genotyped for five polymorphic markers in the PSEN1 region. We then constructed haplotypes using the computer program HAPLO and compared the frequencies between cases and controls. We discovered a novel polymorphism with high heterozygosity on -4752 of the PSEN1 promoter region. A significant association was observed between the -4752C/T polymorphism and late-onset AD. The odds ratio for AD associated with the CC vs non-CC genotype was 1.59 (95% CI= 1.01-2.51), while that of E4 vs non-E4 in APOE gene was 4.41 (95% CI= 2.72-7.16). The C allele was associated with a further increase in the risk of AD in APOE E4 carriers. We found the 12 major haplotypes using five polymorphisms. The distribution pattern was significantly different between cases and controls. The data suggest that genetic variants within the PSEN1 regulatory region might be implicated in the pathogenesis of AD.

Gender differences in the genetic determinants to alcohol dependence. *M. Zatz¹, C. Guindalini¹, S. Scivoletto², RGM. Ferreira¹, A. Nishimura¹, M. Zilberman³, MA. Peluso².* 1) Human Genome Research Center; Dept of Biology, Cidade Univ, Univ of Sao Paulo, Sao Paulo, Brazil; 2) GREA Interdisciplinary Group of Studies on Alcohol and Drugs; Institute and Department of Psychiatry, Medicine Faculty, Univ of Sao Paulo, Sao Paulo, Brazil; 3) Addiction Centre; Foothills Medical Centre, University of Calgary. Calgary, AB, Canada.

Twin and adoption studies suggest that alcoholism is a multifactorial disorder caused by genetic and environmental factors. Among the different possible genes involved in the alcoholism etiology, the X-linked Monoamine Oxidase (MAO) gene, previously reported as involved in the regulation of the central nervous system represents a good candidate. The aim of the present study was to assess whether a functional polymorphism in the promoter region of the MAOA gene was related to alcoholism, comparing patients of both sexes. Ninety alcohol-dependent patients (51 males, 39 females) were selected according to CID 10 criteria. A group of 90 volunteers with a similar ethnic background, sex and age, submitted to the CAGE questionnaire, were selected as the control group. For analysis of the MAO gene genomic DNA was extracted from whole blood and submitted to PCR (polymerase chain reaction) amplification. Genotypic frequencies were compared between the two groups analyzed together and separated according to gender. When the sample was assembled, the allele 1 was significantly more frequent among alcohol-dependent individuals than controls ($p < 0.05$). However, when the two genders were analyzed separately this association was statistically significant only for females ($p < 0.05$). Among males although the allele 1 frequency was still higher, the difference was not statistically significant ($p > 0.05$). The results of the present investigation suggest that there is a greater association between the MAO A promoter polymorphism and susceptibility to alcoholism in Brazilian females than males. In order to validate this hypothesis it will be very important to replicate this study in other populations with similar gender differences in drinking habits.

Epilepsy and Pervasive Developmental Disorders due to a deletion within the AS/PWS critical region. *L. Russo¹, F. Gurrieri¹, C.T. Giordano¹, D. Battaglia², F. Guzzetta², G. Neri¹.* 1) medical genetics, Catholic University, Rome, Rome, Italy; 2) Child Neuropsychiatry, Catholic University, Rome, Rome, Italy.

The 15q11-q13 region is one of the most important candidate regions for epilepsy and PDD. In fact, both cytogenetic and cryptic rearrangements have been found in association with PDD. We screened 180 patients by microsatellite markers analysis in order to identify the real prevalence of duplications or deletions in PDD population. Here we report on a patient with PDD and partial epilepsy: he had an unremarkable prenatal period, normal measurements at birth and marked hypotonia. He had absence of speech, walking disability, seizures, mild dysmorphisms, stereotyped movements and autistic features. Chromosomes and FMR-1 test were normal; parents were consanguineous. These phenotypic features together with the specific pattern of myoclonia seizures prompted us to rule out the Angelman syndrome. Methylation test gave normal results. The analysis with microsatellite markers from the 15q11-q13 genomic region revealed a maternal deletion including the D15S113 and GABRB3 loci. The latter maps in a more centromeric position with respect to the GABRB3 gene; the D15S97 marker, which is intragenic to the GABRB3 gene, was not deleted. In order to define the extension of this deletion we performed Fluorescence in situ hybridisation (FISH) by using BAC-probes mapping between UBE3A and GABRB3 genes. FISH results confirmed the data obtained with microsatellites and showed that UBE3A and GABRB3 genes are not deleted; however, the deletion included ATP10C, a maternally imprinted gene, encoding a putative protein homologous to the mouse aminophospholipid-transporting ATPase Atp10c. These findings suggest that this gene may contribute to the Angelman syndrome phenotype as well as to PDD. In addition, ATP10C isolated deletions or point mutations might be responsible for Angelman-like features in patients who have normal methylation test and no mutation of the UBE3A gene.

Prevalence of *parkin* gene mutations and deletions in idiopathic parkinson's disease. R. Sinha¹, B. Racette², J. Perlmutter², A. Parsian¹. 1) Birth Defects Center, Univ Louisville Hlth Sci Ctr, Louisville, KY; 2) Dept of Neurology, Washington Univ Med Sch, St. Louis, MO.

Mutations in *parkin* gene are a common cause of autosomal recessive young onset parkinsonism (AR-JP) but their role in idiopathic Parkinson's disease (PD) is not clear. Recent studies demonstrate that most young onset PD without family history is not due to mutations in *parkin* but there is less information about the role of this gene in typical onset PD. To determine the frequency of *parkin* gene mutations and deletions in the general population of patients with PD categorized based on family history and age of onset, we screened a sample of PD patients (N= 433) with the age of onset of 22-82 years and normal controls (N= 115) for the previously reported mutations, single nucleotide polymorphisms (SNPs), and deletions in exons 2-12 of the *parkin* gene. A total of 10 mutations in exon 4 (1%) and 2 in exon 7 (0.2%) in the form of heterozygotes were detected in the PD group but none in controls. The SNPs in exon 10 and 11 that cause amino acid changes (arg366trp and asp394asn, respectively) are very rare (1- 5%) and have similar frequencies in PD and normal controls. No mutation was observed in exons 2, 3, 5, 6, 8, 9 or 12. No deletion in exon 3, 4, 5, and 9 were detected in PD or control samples. There was no correspondence between any mutation and family history in any group. We also analyzed the data based on the age of onset of the disease but no correlation was found. Our study is a much larger series than previously published. Therefore, we conclude that these mutations or polymorphisms are not common in idiopathic PD and are not playing any direct role in the development of PD in our patient sample.

Mutations in *LGII* in an Australian family with familial temporal lobe epilepsy with auditory features. R.H.

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Epilepsy is one of the most common neurological disorders, affecting approximately 2% of the population. Identification of families with inherited epilepsies has led to the discovery of several genes involved in both generalized and partial epilepsies. Temporal lobe epilepsy (TLE) is a partial epilepsy syndrome, characterized by seizures that originate in the temporal lobe(s) of the brain. Although TLE is generally considered an acquired disorder, genetics also play a role in the etiology. Several families have now been described with autosomal dominant inheritance of TLE and mutations in the gene *LGII* have recently been reported in seven families with TLE and auditory features. We studied a four generation Australian family with TLE with auditory features, in which five members had epilepsy. Direct sequencing of *LGII* in this family revealed a single base substitution in exon 8 (c.1418C>T). The nucleotide substitution led to a missense mutation at residue 473 of the *LGII* protein (S473L). The S473L mutation was not observed in 96 healthy control subjects. Of the 8 reported mutations in *LGII*, this is only the second missense mutation, all others resulted in truncation of the protein. The serine at residue 473 is highly conserved among all known homologues of *LGII* and is likely to be phosphorylated, according to computer prediction programs. The function of *LGII* remains largely unknown. Further studies in animal models will aid in our understanding of the role of *LGII* in familial temporal lobe epilepsy.

DNA polymorphisms in Oxytocin and 7B2 (SGNE1): evidence for association between polymorphisms and autism spectrum disorder. A. Woodroffe¹, P. Flodman¹, L. Mays¹, J. Lord², S. Holguin¹, C. Modahl¹, K. Osann¹, M.A. Spence¹, D. Fein², M. Smith¹. 1) Pediatrics, University of California-Irvine, Irvine, CA; 2) Psychology, University of Connecticut, Storrs, CT.

Autism spectrum disorder (ASD) is a developmental disorder characterized by impaired communication and social interactions and stereotypic patterns of interest and activity. ASD occurs with a frequency of 1 in 150. Animal studies revealed that Oxytocin (OT) plays a role in species typical communication and social behavior (Insel et al., 1999). Modahl et al., 1998 and Green et al., 2001, reported decreased OT levels and evidence of altered OT processing in autistic males. 7B2 (SGNE1) controls proteolytic cleavage of OT by PC2 and its transportation through and release from secretory granules. Exon 1 encodes a long 5'UTR (Mbikay 2001). We sequenced OT in genomic DNA from 67 ASD subjects and 42 controls. We identified 3 single nucleotide polymorphisms (SNPs) within intron 1 and an insertion/deletion polymorphism (extra A) in intron 2, but no polymorphisms in OT exons. The OT intron 2 sequence represents a potential open reading frame. The insertion of the extra A disrupts this reading frame. Using a Fisher exact test, we showed that the ASD subjects have a genotype with the inserted A less frequently than controls, $p=0.026$ (Fisher's exact test). We found highly significant linkage disequilibrium between the insertion/deletion polymorphism in intron 2 and one of the SNPs in intron 1. The three SNPs in intron 1 were not in linkage disequilibrium with each other. We analyzed the 7B2 gene in 68 ASD subjects and 53 controls. We identified 5 common SNPs: one located in exon 1 (A/T), one in intron 1, two in intron 2 and one in intron 5. A Fisher exact test revealed statistically significant association between ASD status and a genotype that included a T allele in 7B2 exon 1, $p=0.009$. The presence of a T allele in the exon 1 A/T SNP increases the risk of ASD 4-fold. 7B2 maps in 15q12-q13, a region that has been previously reported to play a role in autism. Our current data indicate that 7B2 and OT represent ASD susceptibility loci. [Supported by March of Dimes, Dr. Fein PI and NICHD-HD35458, Dr. Spence PI].

Human substance abuse substance vulnerability loci: identification and initial fine mapping studies. *G.R. Uhl, Q.R. Liu, H. Ishiguro, J.P. Gong, X. Zhu, T. Drgon, J. Draganova, P.W. Zhang, Z. Lin, O. Polesskaya, D. Walther.* Molecular Neurobiology Branch, NIH/NIDA/IRP, Baltimore, MD. 21224.

Convergence of results from association and linkage based genome scans now reveal 12 human rSA chromosomal regions that each contain markers associated with and linked to substance abuse (Uhl, *Trends Genetics*, 2002). These convergent results are very unlikely to have arisen by chance alone. Interesting candidate genes and striking LD are found in several of these regions. rSA1 on chr 3 30Mb has a core region which contains 11 candidate genes including NIMA kinase 6. The core 1Mb region of rSA2 on Chr 3, 175 Mb contains 4 genes including a TRAF2 homolog and PLD1. The rSA3 core contains 6 genes including LEPA and repressor of protein kinase inhibitor genes, while flanking sequences include GABAR genes. rSA7 displays paired SNPs 29 bp apart that both reveal association 4q 125Mb. 12 putative genes lie within a core region, including a putative channel protein. The rSA 8 region contains 8 genes in a core region including PLA2 activating protein and TEK kinase. The rSA 9 region of Chr 10, 106Mb contains a core region with a sortilin-receptor related protein. The 11p rSA10 region near D11S1984 includes a cluster of phosphorylation pathway genes with substantial brain expression, including DUSP8 whose intron 3 variant participates in the abuse-associated haplotypes. The 11p 26 Mb rSA11 region contains >24 genes, including BDNF and GPCR48. The rSA 12 region of 12, 123-24 Mb includes a 2Mb core of high LD with 24 genes including TJ6 and an orphan GPCR. The 13, 94 Mb rSA13 region has a core region of 18 genes including glypican 5, HNRPA1-related protein, and rap2A, several with brain expression. The rSA 14 region of chr 13, centered at 113Mb contains a core of nine genes including phospholipid/ATPase and guanine nucleotide exchange factor genes. The rSA15 region of X, 142 Mb contains 62 genes that include several zinc finger, channel and rab proteins. These features, and the results of initial fine-mapping studies, add to evidence from linkage, association, and linkage disequilibrium patterns to indicate that these regions are strong candidates for substance abuse vulnerability variants.

Tumor necrosis factor α (TNFA) haplotypes modify susceptibility to schizophrenia. *V. Saviouk*¹, *E.W.C. Chow*², *A.S. Bassett*², *L.M. Brzustowicz*^{1,3}. 1) Department of Genetics, Rutgers University, New Brunswick, NJ; 2) Department of Psychiatry, University of Toronto, and Schizophrenia Research Program, Queen Street Division, Centre for Addiction and Mental Health, Toronto; 3) Department of Psychiatry, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark.

The HLA region has been implicated in the etiology of schizophrenia. TNFA is a gene located in the HLA III complex, between HLA I and II, a region known for tight linkage disequilibrium. 337 subjects from 24 Canadian families of Celtic (n=23) and German (n=1) origin were genotyped for two TNFA promoter polymorphisms, G-308A and G-238A. Three families with branches separated by multiple unavailable individuals were divided, producing a total of 27 pedigrees for analysis. Analysis with SIMWALK2 revealed three segregating haplotypes: H1 (-308G, -238G; .04), H2 (-308A, -238G; .78), and H3 (-308G, -238A; .18). Analysis with TRIMHAP revealed association of the H2 haplotype with schizophrenia ($p < 0.01$ with narrow disease definition/dominant inheritance). Pedigrees were stratified into two groups, with and without H2. LOD scores from our prior genome scan were available on 25 pedigrees, and were re-analyzed using these two subsets. The subset with H2 (n=17 pedigrees) produced larger 2-point heterogeneity LOD scores than the total data set at D1S1679 (HLOD=5.9), D3S3045 (3.3), D8S136 (2.4), D9S925 (2.0), and D13S779 (2.7). While most of the increases in the HLODs were small (< 0.3), the HLODs at D3S3045 and D9S925 increased by 0.9 and 1.6, respectively. The subset without H2 (n=8) produced larger HLODs at D1S1609 (2.5), D2S297 (2.3), D5S148 (2.4), and D14S608 (2.2). The magnitude of the average increase in HLOD was larger for this group, ranging from an increase of 1.2 for D1S1609 to an increase of 2.1 for D5S148. While none of the HLODs from this subset reached significance, the magnitude of the scores are noteworthy as this subset represents only 30% of the total sample. These results suggest that TNFA or other loci in the HLA region can modify the susceptibility to schizophrenia caused by other loci in the genome.

Comparing region specific mRNA expression profiles in Progressive Supranuclear Palsy. *P. Rizzu*¹, *V. Bonifati*^{1,2}, *A.H. Rajput*³, *R. Ravid*⁴, *P. Heutink*¹. 1) Clinical Genetics, Erasmus University, Rotterdam, The Netherlands; 2) Department of Neurological Sciences, La Sapienza University, Rome, Italy; 3) University of Saskatchewan, Canada; 4) The Dutch Brain Bank, Amsterdam, The Netherlands.

PSP is a neurodegenerative movement disorder of unknown etiology, neuropathologically characterized by abundant neurofibrillary tangles (NFTs) and neuropil threads consisting of hyperphosphorylated protein tau. To determine whether there are consistent gene expression changes in brain regions of patients affected by Progressive Supranuclear Palsy (PSP) compared to non-affected controls, we isolated total RNA from four brain regions (globus pallidus, caudate, frontal and occipital cortices) from pathologically confirmed PSP cases and controls matched for age and gender. Labeled cRNAs were hybridized to Affymetrix U95 oligo-arrays to determine which genes in the PSP samples show increased or decreased expression level compared to control samples. Conventional molecular biology techniques were applied to confirm findings for individual genes, such as RT-PCR, but also immunohistochemistry and immunoblot with antibodies to investigate protein expression and localization. Approximately half of the total number of genes was expressed in at least one of the four brain regions tested. The overall pattern of gene expression is dissimilar between the PSP and control groups. According to expectations the expression of some of the heat shock proteins, HSP 70 in particular, is increased and genes encoding neurofilament proteins are decreased especially in the globus pallidus. Among the different areas investigated, signal transduction and transcription factors are constantly reported as differentially expressed across PSP and controls. Synaptic, proteolytic and mitochondrial genes account for most of the genes whose expression pattern changes in the globus pallidus. Other differentially expressed genes with unknown function are currently being investigated in more detail. Comparison of gene expression profiles with mouse and cell biological models will allow to identify and characterize cellular pathways/networks that might be potentially involved and play causative roles in PSP.

Family-based association studies of trace amine receptor genes on 6q23.3 in bipolar affective disorder. J.

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There is evidence suggesting a role of trace amines (TA) in the etiology of bipolar affective disorders (BPAD). A functional deficiency of TA has been proposed as a potential etiological factor in depression, increased levels of TA were found to be associated with manic phases of BPAD. The genes for two trace amine receptors, TA-1 and TA-4, are both located on chromosome 6q23.2. Interestingly, this particular chromosomal region has shown evidence for linkage to BPAD in a genome-wide screen (Cichon et al., 2001). This prompted us to search for genetic variants in the TA-1 and TA-4 genes and test them for association with BPAD. Systematic sequencing of the TA-1 and TA-4 genes in 96 control individuals identified two common SNPs in the TA-4 receptor gene (914A/G and 993A/G) and one in the TA-1 receptor gene (1212A/G). TDT analysis of the three SNPs was performed in 118 parent-offspring triads that were partially derived from the genome screen sample. No association between the SNP in the TA-1 receptor gene and BPAD was found. However, we observed a preferential transmission of the 914G allele of the TA-4 receptor gene ($p=0.014$) and a non-significant trend of the 993G allele ($p=0.06$). Replication is clearly necessary to support a possible role of the TA-4 receptor gene in BPAD.

The high-activity catechol-O-methyltransferase *val* allele is a risk factor for autism spectrum disorder. *H. Zhang*¹, *X. Liu*², *C. Zhang*², *E. Mundo*³, *F. Macciardi*³, *C.E. Schwartz*⁴, *R. Michaelis*⁴, *J.J.A. Holden*^{1,2}. 1) Physiology; 2) Psychiatry, Queen's University, Kingston, ON, Canada; 3) CAMH, University of Toronto, Toronto, Ontario, Canada; 4) Center for Molecular Studies, J.C. Self Research Institute, Greenwood Genetic Center, Greenwood, SC, U.S.A.

Autism is a neurodevelopmental disorder characterized by deficits in reciprocal social interaction, verbal and non-verbal communication, and displays of repetitive behaviours. Many of the behavioural problems associated with autism, including hyperactivity and stereotypies, are modulated by catecholamine levels. Since one of the main routes for the breakdown of catecholamines is via catechol-O-methyltransferase (COMT), we examined the COMT gene in autistic families with two or more affected children. The initial test group consisted of 45 families, and a replication set consisted of 81 families. We found an increased frequency of the high-activity *val* allele in autistic individuals randomly selected from both groups of families (1 affected/family) (Set I: $c^2=4.130$, $df=1$, $p=0.042$; Set II: $c^2=9.795$, $df=1$, $p=0.002$), as well as in all affected children (Set I: $c^2=4.797$, $df=1$, $p=0.029$; Set II: $c^2=5.844$, $df=1$, $p=0.016$). This increased *val* allele frequency resulted from a larger frequency of *val/val* children than in the comparison group (*val/val*=24.6%, *val/met*=51.6%, *met/met*=23.8% compared to 16.0%, 52.2%, and 31.8%, $c^2=9.266$, $df=2$, $p=0.010$). Similar increased *val* allele frequencies were seen in both parents (mothers: $c^2=4.301$, $df=1$, $p=0.038$; fathers: $c^2=4.035$, $df=1$, $p=0.045$). To determine whether there were differences in some of the core symptoms of autism in individuals with the different COMT genotypes, information from the ADI-R was used. Although mean ADI-R scores did not differ for individuals with the different COMT genotypes in any of the three domains characterizing autism, there was a tendency for children with at least one *val* allele to have later age of first word ($c^2=3.705$, $df=1$, $p=0.054$). The above results suggest that the COMT gene may play a role in the etiology of autistic spectrum disorders. (Supported by a scholarship to HZ from OMHF and research grants to JJAH from CIHR and OMHF).

Autoantibody repertoires against brain tissue in autism nuclear families. *S.C. Silva¹, C. Fesel¹, A.M. Coutinho¹, M. Barreto¹, C. Marques², T. Miguel², A. Ataíde², C. Bento², G. Oliveira², A.M. Vicente¹.* 1) Instituto Gulbenkian Ciência, Oeiras, Portugal; 2) Hospital Pediátrico de Coimbra, Coimbra, Portugal.

The hypothesis of an autoimmune dysfunction in autism has previously been put forward without, however, compelling evidence of a direct relation to its etiology or pathogenesis. The objective of this study was the identification of immunological traits associated with autism, presenting with a simpler genetic transmission pattern, for subsequent genetic mapping. For this purpose, we have analyzed autoantibody repertoires against brain tissue extract in the serum of 126 autism spectrum disorder (ASD) children, their parents, and 54 controls in the same age range, using a quantitative immunoblotting technique. Multiparametric principal component analysis (PCA) was used for comparison of patients and controls, and the relevant PCA factors were used for heritability estimates in the sample of nuclear families. The results obtained show a significant difference in autoantibody repertoires between ASD children and controls ($p=0.0013$ for both PCA factor 1 and PCA factor 4). For PCA factor 4, analysis of the blot sections revealed a major antigenic contributor with a molecular weight in the range of Myelin Basic Protein (MBP). The correlation coefficients established for this antigenic band between parent/offspring (0.041) and spouse (-0.018) pairs and the heritability estimates ($h^2=0.082$) clearly show the absence of a genetic component for this autoantibody reactivity. Recent studies demonstrate that an autoimmune reaction directed against MBP is a protective physiological response to neuronal injury. We therefore hypothesize that the autoantibody reactivities here described, specifically in ASD patients, are not an etiological factor for autism. Instead, they may represent the immune systems protective response to a previous brain injury, which, in agreement with prevalent hypothesis for autism, might have occurred during neurodevelopment.

Genetic analysis of the Nurr1 transcription factor in Attention Deficit Hyperactivity Disorder. *K.M. Smith¹, R. Mankoski¹, M. Fischer², R. Barkley³, B.A. Navia^{1,4}.* 1) Gen Prog/Sackler Sch GBS, Tufts Univ, Boston, MA; 2) Neuropsych. Sec., Medical College of Wisconsin, Milwaukee, WI; 3) Dept. of Psychiatry, UMass Worcester Med. Cent., Worcester, MA; 4) Neurology, NEMC/Tufts. Univ. School of Medicine, Boston, MA.

Attention Deficit Hyperactivity Disorder (ADHD) is a highly heritable and common disorder that partly reflects alterations in dopamine function. Various studies have shown that genes involved in dopamine signaling and metabolism, including the 7 repeat allele of the D4 dopamine receptor (DRD4) exon 3 VNTR and the 10 repeat allele of the dopamine transporter (SLC6A3) VNTR, contribute to ADHD susceptibility. NR4A2, or Nurr1, is an orphan nuclear receptor implicated in the development of dopaminergic cells of the ventral midbrain. Mice lacking Nurr1 fail to develop dopaminergic cells of the ventral tegmental area (VTA) and the substantia nigra (SN). The dopaminergic cells of the VTA provide innervation to the prefrontal cortex, a signaling system believed to be of major importance to ADHD, suggesting that Nurr1, or one of its targets is a potential candidate gene for ADHD susceptibility. The goals of this study were to identify polymorphisms in Nurr1, and to test their association to ADHD. Database analysis revealed a CA microsatellite repeat polymorphism in the 3' UTR of this gene, which was confirmed by PCR. Two alleles were identified, differing by one repeat length. SSCP screening in 90 individuals revealed rare polymorphisms in intron 5 and the 5'UTR, each present in one individual, and a more common polymorphism -254DC in the promoter region. The CA repeat and -254DC polymorphisms were tested for an association with ADHD in both a case control study of individuals from the Milwaukee Longitudinal Study of ADHD (103 cases and 66 controls), and in 35 sib pairs with ADHD. No association was observed between either polymorphism by case control association or by TDT. Identification of these polymorphisms may aid future candidate gene studies in disorders with altered dopamine signaling, such as Schizophrenia and Parkinson's Disease.

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Mutation in GABA receptor gamma 2 gene is not a frequent cause of idiopathic generalized epilepsies. P.A.

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Recently, two mutations in coding regions in the GABRG2 gene were found in two families with a new epilepsy syndrome called generalized epilepsy with febrile seizures plus (GEFS+) (Baulac et al., 2001) and childhood absence epilepsy (CAE) and febrile seizures (FS) (Wallace et al., 2001). There is remarkable intrafamilial phenotypic variation in these families and some of the phenotypes are also found in common forms of idiopathic generalized epilepsy (IGE). This suggested to us that mutations in the same gene could be responsible for other types of IGE. We examined the GABRG2 gene to check whether it could be responsible for other types of common forms of IGE, with and without febrile seizures. We sequenced the whole coding sequence including splice sites of the GABRG2 gene in 44 patients with different forms of IGE, including CAE (with and without febrile seizures) and Juvenile Absence Epilepsy. No mutation was found. It indicates that GABRG2 gene is not a frequent cause of idiopathic generalized epilepsies. [Support: DK31775, NS27941, MH48858].

Linkage disequilibrium analysis of 6q13-q26 schizophrenia candidate genes. A.R. Sanders¹, M. Martinez², L. Martinolich¹, E.B. Carpenter¹, J. Duan¹, B.J. Mowry³, D.F. Levinson⁴, R.R. Crowe⁵, J.M. Silverman⁶, P.V. Gejman¹. 1) Schizophrenia Genetics Research Program, Univ of Chicago, Chicago, IL; 2) INSERM EMI6, Méthodologie Statistique et Epidémiologie Génétique des Maladies Multifactorielles, Evry, France; 3) Queensland Centre for Schizophrenia Research, Wolston Park Hospital, Univ of Queensland, Brisbane, Queensland, Australia; 4) Dept of Psychiatry, Univ of Pennsylvania School of Med, Philadelphia, PA; 5) Dept of Psychiatry, Univ of Iowa College of Med, Iowa City, IA; 6) Dept of Psychiatry, Mount Sinai School of Med, New York, NY.

We report generation of polymorphisms of chromosome 6q13-q26 candidate genes and linkage disequilibrium (LD) analysis thereof to schizophrenia. The chromosome 6q13-q26 region has been implicated as harboring a schizophrenia susceptibility gene (SCZD5) in multiple independent family collections, and most recently, in an over 1,000 affected sibling pair (ASP) replication effort. We have examined *in silico* over 1,000 positional candidate genes mapping to chromosome 6q13-q26 to prioritize their examination, based upon their known functions and putative relationships to schizophrenia etiology. Thereby, we have selected 18 of these genes (ACAT2, CNR1, ESR1, GABRR1, GABRR2, GRIK2, GRM1, HTR1B, HTR1E, IGF2R, LPA, NMBR, NR2E1, PARK2, SLC22A3, SLT, SOD2, and TCP1) to: (1) scan for several informative SNPs by published scans, database comparisons, and denaturing gradient gel electrophoresis (DGGE); (2) genotype these SNPs in the National Institute of Mental Health (NIMH) Genetics Initiative I schizophrenia families by means of DGGE, RFLP, or fluorescence polarization (FP) methods (55 SNPs examined to date); (3) examine for evidence of LD to schizophrenia; (4) increase the statistical power for SNPs yielding promising LD test results by additionally genotyping other available schizophrenia families (for a total of 837 individuals genotyped); and (5) generate additional SNPs to then examine for LD with the more promising positional/etiological candidate genes. We have obtained in our preliminary results nominally significant LD for marker(s) of the following genes: GRIK2, HTR1B, HTR1E, and SOD2.

Mutation screening and association study of LIMK1 in Autism. *M. Shinya*¹, *A. Yamamoto*², *A. Oka*^{1,2}, *M. Uchida*¹, *E. Tokubo*¹, *R. Sato*¹, *H. Inoko*^{1,2}. 1) Sch Medicine, Tokai Univ, Isehara, Japan; 2) JBIRC, Tokyo, Japan.

Autism is a neurodevelopmental disorder that characterized by marked social deficits, delay and deviance in language development and communication skills, and a restricted range of stereotypical, repetitive behaviors and limited interests. Autism is considered to arise on the basis of complex genetic predisposition. Several studies have indicated the presence of an autistic disorder susceptibility locus within the distal long arm of Chromosome 7. LIMK1, located on 7q11.23, encodes a protein tyrosine kinase with two repeats of the LIM/double zinc finger motif, and it is highly expressed in brain. LIMK1 regulates actin cytoskeletal reorganization through phosphorylation and inactivation of cofilin, a protein that is required for turnover of actin filaments. Thus, we expected that LIMK1 may prevent neurons from making their proper connections and is linked to the neurological disorder of Autism. We have screened LIMK1 coding region for sequence variants. Several polymorphic makers were identified. To further investigate transmission disequilibrium between LIMK1 and autism, a family based association study was performed for these markers in 100 AGRE autism trios.

Genomic Structure and Mutation Analysis of Semaphorin 3C. *W. Gong*², *M.L. Colavincenzo*², *E. Goldmuntz*³, *D.A. Driscoll*^{1, 2}. 1) Dept Obstetrics/Gynecology, University of Pennsylvania; 2) Div. Human Genetics and Molecular Biology and; 3) Div. Cardiology, The Children's Hospital of Philadelphia, Philadelphia, PA.

Semaphorin 3C (SEMA3C), a secreted glycoprotein involved in neuronal guidance, is expressed in the cardiac outflow tract in mice. Mice deficient in *Sema3C* display interrupted aortic arch (IAA) and persistent truncus arteriosus (PTA). The cardiac defects observed in *Sema3c* mutant mice are strikingly similar to that seen in the 22q11 deletion syndrome. However, SEMA3C maps to 7q21-q31 and therefore, is considered a promising new candidate gene for IAA and PTA, prompting us to investigate the role of SEMA3C in humans. Northern blot analysis demonstrated a unique 5.2 Kb transcript in fetal and adult heart, especially in the aorta. To determine the genomic structure of SEMA3C we compared the human cDNA sequence and the corresponding genomic sequence of human chromosome 7q31. This revealed that SEMA3C consists of 18 exons spanning 5,177bp cDNA sequence. We identified a gap between exons 4 and 5. The fragment spanning the gap was amplified and sequenced, which completed the genomic sequence of SEMA3C and allowed us to begin mutational studies to examine the role of SEMA3C in human cardiac development. Twenty sets of primers flanking all coding regions, exon-intron junctions, 5'UTR and partial 3'UTR were designed. Mutation screening was performed by confirmation-sensitive gel electrophoresis (CSGE) followed by direct sequencing. For the initial screening, we analyzed genomic DNA from 11 probands with IAA and up to 70 normal controls. We identified 12 base changes including one deletion and 11 base substitutions. Five are single nucleotide polymorphisms (SNPs). The most interesting change is an A/G substitution at 44 bp 3' to the splice donor site of exon 1, found in one patient, but not 140 normal chromosomes. This variant is located in a conserved region and predicted to create a potential splice donor site GT. Further experiments are in progress to determine the consequence of this change and to determine if SEMA3C mutations are associated with PTA in humans.

Association study of MTHFR polymorphisms and congenital heart defects. *D.A. Driscoll^{1,2,5}, T.A. Boland⁵, J.A. Campanile⁵, E. Levin², K. Brown³, E. Goldmuntz², L. Mitchell^{4,5}.* 1) Depts Obstetrics/Gynecology; 2) Pediatrics; 3) Pharmacology; 4) Clinical Epidemiology & Biostatistics, Univ Pennsylvania; 5) Division Human Genetics & Molecular Biology, Children's Hospital Philadelphia, Philadelphia, PA.

Two small case-control studies have reported an association between CHD and the MTHFR C677T polymorphism. We sought to further investigate the association between MTHFR and CHD, in families ascertained through a child with either a conotruncal (CT) or a left-sided (LS) cardiac defect. Here, we report on our analyses of data from 349 families (253 CT, 96 LS), which represents the largest family-based genetic association study of CHD, to date. The transmission disequilibrium test (TDT) was used to assess the association between CT and LS, and these two polymorphisms.

MTHFR Polymorphisms	C677T	A1298C*
#Informative transmissions	265	273
#Transmissions of T and C*	145 (54.7%)	130 (47.6%)
p-value	0.12	0.43

Analysis of the data from CT and LS families combined, shown in table, provided no evidence that either the C677T or A1298C polymorphisms were significantly related to the risk of these cardiac malformations. Analysis of data from each subgroup, CT and LS alone, also provided no evidence that either MTHFR polymorphism was significantly related to these specific cardiac defects. These results are in contrast to previous reports. However, our study has the advantage of a relatively large study population that allowed for subgroup analyses, and employed the TDT which, unlike case-control studies, is not subject to bias arising from population stratification.

Hemochromatosis-associated genotypes are not associated with early markers of atherosclerosis. *P.J. Bridge¹, L.M. Yunker¹, M.E. Hyndman², M. Fung², J.S. Parboosingh¹, T.J. Anderson².* 1) Molecular Diagnostic Lab, Alberta Children's Hospital, Calgary, AB, Canada; 2) Medicine, University of Calgary, AB, Canada.

Background: Patients with hereditary hemochromatosis (HH) suffer from iron accumulation and may be at increased risk of cardiovascular disease. The majority of HH patients are homozygous for the C282Y mutation in the HFE gene while a small number have some other combination of C282Y, H63D, S65C or unidentified mutations. Excess iron is known to result in increased oxidative stress and lipid peroxidation. Increased oxidative stress is thought to play a role in development of atherosclerosis and endothelial dysfunction, and some studies have shown an association between HFE genotype and cardiovascular disease. In an attempt to investigate how early such an effect might occur, we investigated the effect of three HFE mutations on presymptomatic atherosclerosis markers, brachial artery endothelial function measured by flow-mediated vasodilation (FMD) and carotid intima medial thickness (CIMT).

Methods and Results: We examined the association of the C282Y, H63D and S65C mutations with FMD and CIMT in 484 healthy middle-aged Caucasian men participating in a prospective study of endothelial function (mean age 45 yrs). Significant differences in ferritin saturation versus genotype indicated that the HFE mutations were penetrant in these subjects. The distribution of genotypes for each of the mutations were in Hardy-Weinberg equilibrium. No significant difference in the clinical markers was observed between the different HFE genotypes, however, there was a significant association between increased ferritin saturation and increased CIMT ($p=0.002$).

Conclusion: These results suggest that cardiovascular risk may be greater in individuals with greater ferritin saturation (and that mutant HFE genotypes are one, but not the only, contributor to absolute ferritin saturation levels). The effect of these mutations on atherosclerosis requires further investigation.

LOX-1 polymorphism as a susceptibility genetic marker for atherosclerosis. *R. Mango¹, G. Contino¹, F. Clementi², P. Borgiani¹, A. Botta¹, G.B. Forleo², A.M. Nardone¹, G. Chiricolo², S. Guarino¹, M.R. D'Apice¹, A. Romeo¹, M. Federici², R. Lauro², J.L. Mehta³, F. Romeo², G. Novelli¹.* 1) Dept Biopathology, University Tor Vergata, Rome, Italy; 2) Dept Internal Medicine, University Tor Vergata, Rome, Italy; 3) Dept Internal Medicine, University of Arkansas for Medical Sciences, Little Rock, USA.

Atherosclerosis is the leading cause of mortality in industrialized countries accounting for up to 50% of deaths. Atherosclerosis is the principal process contributing to the pathogenesis of coronary artery disease (CAD), cerebral infarction, and peripheral vascular disease. A large number of risk factors such as hypertension, hypercholesterolemia, diabetes, obesity, smoking and shear stress leads to endothelial activation and/or dysfunction, which elicit a series of cellular interactions that culminate in atherogenesis. Several biochemical and functional studies suggest that a lectin-like receptor for oxidized low-density-lipoprotein (ox-DL), termed LOX-1, and may be involved in atherogenesis. LOX-1 is a type-II membrane protein belonging to the C-type lectin family. A recent linkage study performed in a mouse model, identified LOX-1 as candidate susceptibility gene for human atherosclerosis. In order to investigate the role of LOX-1 in human atherosclerosis susceptibility, we screened a group of 164 Italian individuals with angiographic CAD phenotype (CAD; n=88) or without any angiographically demonstrable disease (CAD-free; n=76). We characterized five different SNPs (SNP 1-5) at the LOX-1 locus in this population. We demonstrated that SNP4 (A to G transition) correlates with CAD with a high degree of specificity ($X^2 = 7,37$; $P, 0.007$; 1df). This is the first report of LOX-1 SNPs and atherosclerosis. We believe that LOX-1 may be a candidate gene for atherosclerosis and endothelial dysfunction in response to ox-LDL. Work supported by the Italian Ministry of University and Research (MIUR).

GENOMIC STRUCTURE OF THE HUMAN GAS6 GENE AND IDENTIFICATION OF ALLELIC

VARIANTS. X. Munyoz¹, L. Sumoy², P. García de Frutos³, N. Sala¹. 1) Molecular Genetics, CGMM-IRO, Barcelona, Spain; 2) Centre de Regulació Genòmica, Laboratori de Microarrays, Barcelona, Spain; 3) Laboratorio de Investigaciones Biomédicas, Hospital Universitario Virgen del Rocío, Sevilla, Spain.

Gas6 (growth arrest-specific gene 6 product) is a new vitamin K dependent protein, structurally related to the blood anticoagulant protein S. Characterization of the *gas6* knockout mice confirms the importance of this protein in hemostasis and thrombosis, through its role in platelet aggregation and secretion pathways. Since little is known about the human *GAS6* gene, the purpose of the present study was to determine its intron-exon structure and to analyze it for the presence of allelic variants that could be associated with cardiovascular disease. **Methods:** The localization and sequence of *GAS6* exons and introns has been determined *in silico* by comparing the *GAS6* cDNA sequence with the whole human genome sequence using BLASTn program. Characterization of the *GAS6* exons and their intron flanking sequences allowed us to design oligonucleotides for their specific amplification by PCR. *GAS6* amplified fragments from a minimum of 10 control DNA samples were analyzed for the presence of sequence variants by Single Strand Conformation Polymorphisms (SSCPs) analysis and DNA sequencing. **Results:** Comparison of the *GAS6* cDNA with the recently reported human genome sequence allowed us to localize 15 *GAS6* exons and 14 introns in approximately 45 Kb of DNA. SSCP analysis allowed the identification of 6 different polymorphic single nucleotide substitutions (SNPs). Three of them are localized in exons 11, 12 and 14 and appear to be neutral since they do not modify the encoded amino acid. The other SNPs are in introns 7 and 8 and their effect has not yet been analyzed. **Conclusions:** The homology between protein S and *GAS6* is maintained at the genomic level with *GAS6* having, like PROS1, 15 exons. The identification of 6 different SNPs in *GAS6* will be very useful in future association studies aimed to analyze the role of *GAS6* in human disease. **We thank ISCIII 01/1468 and SAF 2001-1059-C02 for grants.**

Genetic factors of stroke with hyperhomocystinemia of young population in Taiwan. *J. Hou.* Medical Genetics, Chang Gung Children's Hospital, Taoyuan, Taiwan.

From the natural history in typical homocystinuria, high plasma homocystine (HCY) levels in such patients constitute a significant risk factor for vascular disease, such as thrombo-embolic complications at an early age. Similar findings also exist in young population with moderate or secondary hyperhomocystinemia (HH). The defective methylenetetrahydrofolate reductase (MTHFR) activity due to the homozygous presence of a thermolabile variant (t-MTHFR) of the enzyme has been recently described as a major cause of moderate HH. To evaluate the role of the t-MTHFR allele as a predisposing factor for early-onset stroke in HH children and young adult, we compare the prevalence of homozygosity and compound heterozygosity of this gene (C677T, A1298C, and T1317C) in young stroke patients (after excluding other etiologies of stroke) with reference populations. Totally 120 young patients (ranged from 6 years to 45 years) were evaluated, 30 % were homozygous for the t-MTHFR allele (C677T: 22%, and A1298C: 8%), compared with 6 % (3% and 2%, respectively) of the reference population ($p < 0.005$); 15 % were compound heterozygous for the allele (C677T plus A1298C), compared with 2 % of a reference population ($p < 0.005$). There was a significant increment of plasma total HCY concentration in subjects with homozygotes and compound heterozygotes compared with those with heterozygotes or negatives (homozygous for the wild-type allele), and controls with no history of stroke. These data suggest that homozygosity or compound heterozygosity for the t-MTHFR allele is associated with raised HCY levels in children and young adult, and is a risk factor for early-onset stroke. MTHFR gene mutation has been documented as an important cause of HH in vascular diseases, and the impact to general health care of this unconventional risk factor of vascular disease has been met. These patients can be treated with folic acid, vitamin B6 and vitamin B12, thus the early diagnosis and early prevention are beneficial for this special population.

Longitudinal Expression Profiling and Analysis of Exercise-Responsive Genes in Type-2 Diabetics. *D.S. Hittel¹, W.E. Kraus², E.P. Hoffman¹*. 1) Center for Genetic Medicine, Children's National Medical Center, 111 Michigan Ave, NW Washington DC 20010; 2) Center for Living, Duke University Medical Center, 1300 Morreene Road Durham, NC 27710.

Aerobic exercise is an important adjunct to the treatment and prevention of the many negative physiological outcomes of type-2 diabetes. While there is substantial anecdotal evidence in support of a favorable relationship between cardiovascular fitness and physical activity there are few consistent models of the molecular mechanisms which underlay this relationship. The Studies of a Targeted Risk Reduction Intervention through Defined Exercise (STRRIDE), is a randomized controlled clinical trial of different doses and intensities of exercise in a structured exercise program. The goal of this study is to more clearly define the relationship between the amount and intensity of exercise and improvements in cardiovascular health. Participants (equal numbers of men and women; 30% minority) are typically 40-65 years of age, moderately overweight with fasting hyperinsulinemia. We hypothesize that the physiological adaptations of skeletal muscle to exercise are primarily responsible for the benefits of exercise training on cardiovascular health and that the differential expression of genes and their protein products in is largely responsible for these changes. Skeletal muscle biopsies were obtained from STRRIDE participants upon entry into the study, after 6 months of exercise training and after 2-weeks of de-training. Expression profiles for several thousand genes were obtained from these biopsies using the Affymetrix DNChip platform. These expression profiles were filtered for genes whose transcript levels which changed consistently and significantly ($P < 0.05$) more than two fold after training and/or de-training relative to the control. These genes were subsequently grouped into functional classes and representative genes selected for confirmation by QMF-RT-PCR, western blot and immuno-staining. Many of these exercise-responsive genes participate in glucose metabolism, insulin signaling, fibrinolysis and other physiological processes that are negatively affected in type 2 diabetics.

Large-scale comparison of SNP and haplotype frequencies in 100 genes between 590 candidates for lipid-lowering therapy and 21 individuals from the general population. *C.J. Messer, J.A. Schneider, M. Pungliya, J.Y. Choi, A.E. Anastasio, K. Parks, R. Jiang, J.C. Stephens.* Genaissance Pharmaceuticals, New Haven, CT.

We compared SNP and haplotype frequencies in ~100 candidate genes relating to cholesterol, energy and drug metabolism and inflammation in two population groups. The first group included 590 individuals of European ancestry who are candidates for lipid-lowering therapy based on National Cholesterol Education Program guidelines for LDL-cholesterol, and the second group included 21 unrelated individuals of European ancestry from the general population. We have conducted high-throughput SNP and haplotype discovery on the 21 individuals from the general population, and are currently investigating the 590 individuals as part of a separate pharmacogenetic study of statin response. DNA sequencing was performed on several functional gene regions, and haplotypes were inferred on non-singleton SNPs using an Expectation-Maximization algorithm. Within the ~100 candidate genes, we found over 1000 SNPs in the 21 individuals from the general population, and nearly 3000 SNPs in the 590 individuals considered in the statin study. The number of haplotypes inferred was slightly less than the number of non-singleton SNPs for the group of 21 individuals from the general population, and slightly more than the number of non-singleton SNPs in the 590 candidates for lipid-lowering therapy. Five of the genes considered contained SNPs with more than 20% frequency difference between the two population groups. In addition, two of these genes also showed haplotypes with comparable frequency differences. A more detailed analysis of the association between SNPs and haplotypes with baseline clinical measurements is required to confirm these results; however, these genes are potential candidates for influencing susceptibility to high LDL-cholesterol.

Dilated Cardiomyopathy and the Microsatellite Polymorphism of Heme Oxygenase 1. *J.M. Pohorence Ferguson, Y. Liu-Stratton, G. Cooke, P.F. Binkley.* Davis Heart and Lung Research Institute, The Ohio State University, 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. Oxidative stress is a major source of damage in both ischemic cardiomyopathy (ICM) and dilated cardiomyopathy (DCM). Because a loss of HO-1 activity increases tissue susceptibility to oxidative damage, the hypothesis of this study was that protective actions of HO-1 are inhibited in patients with cardiomyopathy due to a microsatellite polymorphism in the promoter region of HO-1 that reduces transcriptional activity. Accordingly, sequence analysis was performed using DNA extracted from 24 patients with ICM and 24 patients with DCM to identify the presence of this microsatellite polymorphism (L) which consists of ³ 29 dinucleotide repeats. The frequency distribution for the presence of at least one L allele is shown below. These data indicate that patients with DCM possess the L allele in a significantly (Chi square $p = 0.04$) higher proportion than ICM patients. Therefore, despite the absence of significant coronary disease, patients with DCM may be susceptible to progressive ventricular dysfunction due in part to an inability to protect against myocardial oxidative stress.

Frequency Distribution for L

Allele

Etiology	No L	L Present
ICM Number	17	7
ICM Percent	71%	29%
DCM Number	10	14
DCM Percent	42%	58%

Methionine Synthase SNP and functional assays of cardiovascular risk. *L.M. Yunker¹, M.E. Hyndman², M. Fung², T.J. Anderson², J.S. Parboosingh¹, P.J. Bridge¹.* 1) Medical Genetics, University of Calgary, Alberta, Canada; 2) Medicine, University of Calgary, Alberta, Canada.

Background: Functional variations in folate metabolism alter total homocysteine and folate levels, independent risk factors for developing cardiovascular disease. Methionine Synthase (MTR), a key enzyme in folate metabolism, catalyzes the conversion of homocysteine to methionine. An A>G SNP at nucleotide 2756 results in a Gly to Asp change. The exact physiological effect of this variant on cardiovascular risk and folate metabolism is yet to be elucidated. Hyndman et al. (*Am J Cardiol* 86:1144-6, 2000) showed that of 109 individuals who had previously suffered a cardiac event, those carrying the MTR 2756 G allele were 3.4 times less likely to suffer a recurrent event suggesting that this variation is somehow protective. **Methods/Results:** We analyzed the effect of the MS 2756 SNP (A>G) on folate levels and surrogate markers of atherosclerosis. These included brachial artery endothelial function (flow-mediated vasodilation, FMD) and carotid intima-medial thickness (CIMT). We studied 360 middle-aged males (mean age 45 years) without overt atherosclerosis participating in the FATE study (Firefighters And Their Endothelium). Using an internal primer extension method 262 individuals were found to be A/A, 90 A/G and 8 G/G giving allele frequencies of 0.85A and 0.15G. Preliminary analysis showed no association between the MS2756 genotypes and folate levels. There appears to be a trend towards decreased CIMT thickening (beneficial) in individuals who are either carriers or homozygous for the G allele (A/A .628mm, A/G .606mm, G/G .583mm) however the difference between the average mean CIMT in A/A versus A/G and G/G individuals is currently not significant (p=0.126). **Conclusion:** We are unable to demonstrate a clear relationship between MTR2756 and atherosclerosis as assessed by brachial endothelial function and CIMT. Given the allele frequency, and that we have a further 850 participants already available to study, we will continue to try to clarify the relationship between the MS2756 genotype and presymptomatic cardiovascular markers.

Folate resistant Neural Tube defects associated to MTHFR A1298C mutation. *M.A. Orera¹, A. Zuniga², J. Ubeda³, N. Villanueva³, E. Dulin³, M.D. Lillo¹, C. Lostau¹.* 1) Genetics, H. Gregorio Maranon, Madrid, Spain; 2) B. Molecular. H de la Ribera, Alzira Valencia; 3) Biochemistry. H. Gregorio Maranon.

INTRODUCTION: Neural tube defects are thought to be of multifactorial origin involving a number of genetic and environmental factors. It is not clear which is the contribution of each risk factor that is being investigated and it appears that their impact could be different among the populations studied. The prevalence of NTD shows wide regional and ethnical variation and the recurrence rate is somehow related to the basal rate. In Spain NTD prevalence is 0.7-1.2 per 1000, and the recurrence rate is 1-2 %. Periconceptional folate administration reduces both the occurrence and recurrence risks, indicating a potential role of folate metabolism genes in the development of NTD. There are two mutations of the Methylene tetrahydrofolate reductase gene, C677T and A1298C that are generally considered risk factors in the development of NTD. **MATERIAL AND METHODS:** We have followed the gestational outcome of 83 women with a previous pregnancy complicated with a NTD. All of them were treated preconceptionally with 5 mg/day of Folic Acid. Two non related patients had a NTD in the following pregnancy: Patient 1 is 36 years old and had a first pregnancy not treated with Folic Acid complicated with open spina bifida. She had preconceptional prophylaxis with Folic Acid in the second pregnancy, but the gestation produced a fetus with anencephalia. Patient 2 is 34 years old and she has a history of two pregnancies treated preconceptionally with 5mg/day Folic Acid, with open spina bifida ascertained by ultrasound. Basal plasma Homocysteine and Folic Acid levels were within normal limits for both patients. MTHFR mutation analysis revealed that patient 1 was homozygous for the A1298C mutation and patient 2 was heterozygous. **DISCUSSION:** We present two patients with folate resistant DTN and both of them have the A1298C mutation in the MTHFR, without apparent disruption of folate metabolism. We suggest that this mutation might be related to an independent risk factor that is not modulated by Folate.

Deletions in only one of the two variants of the 4q subtelomere cause Facioscapulohumeral muscular dystrophy: b-satellite the culprit? *R.J.L.F. Lemmers¹, P. de Kievit¹, L. Sandkuijl¹, G.W. Padberg², G.J.B. van Ommen¹, R.R. Frants¹, S.M. van der Maarel¹.* 1) Human & Clinical Genetics, LUMC, Leiden, The Netherlands; 2) Department of Neurology, UMCN, Nijmegen, The Netherlands.

Subtelomeres define the proterminal ends of chromosomes and are composed of patchworks of large polymorphic segments of DNA that are present on many chromosomes. Ectopic recombination between subtelomeres serves interindividual gene family diversity, but may also underlie human disease and mental retardation. Probably the best-studied human subtelomeres are 4qter and 10qter, which are almost identical over a distance of 100-500kb. Partial deletions in the subtelomeric D4Z4 repeat array on 4qter cause Facioscapulohumeral muscular dystrophy (FSHD), while identical deletions when affecting the 10qter repeat are non-pathogenic. Likely, the partial deletion on chromosome 4 results in a transcriptional deregulation of critical genes on 4qter, similar to position effect variegation. Recently, we identified two distinct 4q alleles, 4qA and 4qB, based on a 10kb polymorphic segment directly distal to D4Z4. While both alleles are equally frequent in the population, we find that FSHD is uniquely associated with partial D4Z4 repeat array deletions on 4qA alleles. Recombination is suppressed between 4qA and 4qB in the DNA segment proximal and distal to D4Z4, but not within D4Z4 repeat array proper. Moreover, since 4qA and 4qB alleles are equally susceptible to rearrangements, a functional difference must underlie the association of 4qA with FSHD. The most prominent feature that may explain this functional difference is the presence of a 6.2kb-sized b-satellite repeat array, which is absent on 4qB. The 4qter region is the first example of an intrinsically benign subtelomeric allelic variation predisposing to disease.

Hypomethylation of the D4Z4 repeat array in Facioscapulohumeral muscular dystrophy alleles. *P.G.M. van Overveld¹, L. Sandkuijl¹, L. Enthoven², G.W. Padberg³, R.R. Frants¹, S.M. van der Maarel¹*. 1) Human Genetics, LUMC, Leiden, Netherlands; 2) LACDR, Division of Medical Pharmacology, Leiden University, Leiden, The Netherlands; 3) Department of Neurology, UMCN, Nijmegen, The Netherlands.

Autosomal dominant Facioscapulohumeral muscular dystrophy (FSHD) progressively affects the facial, shoulder and upper arm muscles. FSHD is associated with partial deletion of the polymorphic D4Z4 repeat array residing in the subtelomere of chromosome 4q. Normally, the array varies between 11-150 units, while FSHD patients carry alleles of 1-10 units. A highly homologous array on chromosome 10qter is not associated with disease. The heterochromatic properties of D4Z4 have led to the hypothesis that FSHD is caused by a position effect variegation (PEV)-like mechanism in which partial deletion of the D4Z4 repeats results in a local change in chromatin structure and consequently in a transcriptional deregulation of one or more genes on 4qter. To obtain evidence for a PEV-mediated pathology, we studied the methylation of the D4Z4 repeat array applying a CpG methylation-sensitive chromosome 4-specific DNA digestion assay on DNA isolated from peripheral blood lymphocytes and skeletal muscle tissue. In those individuals with a translocated 10-type array on chromosome 4 and thus carrying only one 4-type and three 10-type D4Z4 repeat arrays, we were able to quantify the methylation of a single healthy or disease allele. We showed that some, but not all CpG methylation-sensitive restriction sites were significantly hypomethylated in FSHD alleles compared to control alleles. Although FSHD patients carry hypomethylated disease alleles, we did not observe a linear relation between the methylation and the length of the D4Z4 repeat. Apparently, a considerable variation in methylation is tolerated but, if upon partial deletion of the D4Z4 repeat array the methylation decreases below a critical threshold, the disease develops. The observed hypomethylation strongly supports a model for FSHD in which a deletion-mediated local chromatin decondensation alters gene expression of 4qter.

Ethnic differences in the expression of neurodegenerative disease: Machado-Joseph disease in Africans and Caucasians. A.A. Singleton¹, S.H. Subramony³, D. Hernandez², S. Smith-Jefferson⁵, J. Hussey⁴, K. Gwinn-Hardy¹, T. Lynch^{6,7}, O. McDaniel⁸, J. Hardy^{2,9}, M. Farrer⁴, A. Singleton². 1) Neurogenetics, NIH/NINDS, Bethesda, MD, USA; 2) Neurogenetics, NIH/NIA, Bethesda, MD, USA; 3) University of Mississippi Medical Center, Neurology Department, Jackson, Mississippi, USA; 4) Laboratories of Neurogenetics, Department of Neuroscience, Mayo Clinic, Jacksonville, Florida, USA; 5) Division of Medical Genetics, Department of Preventive Medicine, University of Mississippi Medical Center, Jackson, Mississippi, USA; 6) Department of Neurology, Columbia University, New York, USA; 7) Department of Neurology, Mater Misericordiae Hospital, Dublin, Ireland; 8) University of Mississippi Medical Center, Surgery Department, Jackson, Mississippi, USA; 9) Department of Neurology, Reta Lila Weston Institute of Neurological Studies Royal Free And University College London Medical School London, United Kingdom.

We describe several families of African origin with SCA3/Machado-Joseph disease gene expansions. In these cases, the phenotype ranges from ataxia with parkinsonian signs to a syndrome clinically almost indistinguishable from idiopathic, L-dopa-responsive Parkinson's disease. In contrast, these parkinsonian phenotypes are rare in those of European descent. Haplotype analysis shows that these African families do not share a common founder, thus a cis-acting element in the promoter is unlikely to be responsible for these unusual presentations. We suggest that trans-acting factors are responsible for the variable phenotype and discuss the implications of diseases showing racially different expressivities.

Genetic factors studied in a family with autosomal dominant autoimmune disease. *C. Spangenberg, T. Trost, S. Fees, E. Lausch, D. Prawitt, F. Zepp, J. Spranger, B. Zabel.* Dept Pediatrics, Univ Mainz, Mainz, Germany.

Systemic lupus erythematosus (SLE) and LE-like conditions are part of a heterogeneous group of chronic disorders involving the immune system. The spectrum of clinical symptoms include organ specific changes of skin and kidneys. It is believed that the disease results from a failure of regulatory mechanisms of the immune system. In this respect the conditions are often associated with the production of autoantibodies which are directed against several nuclear antigens like double-stranded DNA, RNA, and nucleic acid-binding proteins such as histones and Sm. Several genes and chromosomal loci have been linked to different forms of LE. For some of these genes - e.g. DNase1, the complement components C1q, C2, and C4 - mutations could be found in LE patients. However, due to the multifactorial nature of the disease genetic events resulting in development of LE are not yet understood in most cases. Genetic linkage studies in large families as well as the study of LE conditions in animal models have led to the identification of disease-associated candidate genes, thus helping to dissect the role of single genes in complex human diseases. We present a three-generation LE family showing autosomal dominant inheritance of the disease phenotype. A genetic linkage analysis of this family is in progress. We have started to look for co-segregating haplotypes in four affected and several unaffected family members. Highly polymorphic markers were chosen for their proximity to genes that have previously been shown to be mutated in SLE patients (like DNase1) or that have been associated with SLE in genetically engineered mice (like serum amyloid protein, SAP). Genes identified by our linkage studies will be subject of mutational analysis.

Genetics of Autism: Identification of Chromosomal Abnormalities and Susceptibility Loci on Chromosome 15q in Autism Families. *C.D. Kashork, D.W. Stockton, T. Sahoo, D. Bercovich, L.G. Shaffer, A.L. Beaudet.* Human & Molecular Genetics, Baylor College of Medicine, Houston, TX.

Classic autism or autistic disorder is a neuro-developmental disorder characterized by early childhood onset of: impaired social interaction and communication capabilities, deficits in language development, and patterns of stereotypical-repetitive behavior. A high concordance in identical twins has been interpreted to imply a strong genetic component in the etiology of autism, although we suggest that de novo epigenetic defects might equally well underlie this concordance. Numerous studies suggest that a number of chromosomal regions harbor susceptibility loci for autism, and the imprinted domain on chromosome 15q11-q13 remains a significant one. Abnormalities of chromosome 15 and fragile X mutations have been the most frequent genetic abnormality in autistic patients. To assess the frequency of chromosome aberrations involving the Prader-Willi/Angelman syndrome critical region on 15q11-q13, we carried out interphase FISH on lymphoblastoid cell lines from 56 probands with autism [obtained from the Autism Genetic Research Exchange (AGRE) resource]. To determine linkage and allele sharing for loci in the 15q11-q13 interval, we are genotyping all 56 of these families for six polymorphic microsatellite markers near the *UBE3A* gene in the PWS/AS critical interval. We detected an interstitial duplication of the chromosome 15q11-q13 region using FISH in two affected sibs in one family. The duplication was present in the phenotypically normal mother, presumably on her paternal chromosome. The authenticity of this duplication event was confirmed by microsatellite marker analysis. Preliminary analysis of linkage data for loci in this interval is underway. We are examining the hypothesis that a substantial fraction of autism might be caused by paternal or maternal epigenetic defects of 15q11-q13.

Neurocognitive Function in Williams Syndrome (WMS): Evidence for Genetic Imprinting. *H.J. Antoine¹, H. Wijesuriya¹, F. Rose², U. Bellugi², D. Mills³, J.R. Korenberg¹*. 1) Med Gen, Cedars-Sinai, LA, CA; 2) Salk Inst., San Diego, CA; 3) Emory Univ., Atlanta, GA.

WMS is caused by the deletion of 18 genes on chromosome band 7q11.23. Individuals with WMS exhibit global cognitive deficits with striking dissociations characterized by relative strengths within the verbal domain and weaknesses within the visual-spatial domain. Despite a rather uniform pattern of cognitive functioning in WMS, there is considerable variability in the range of cognitive abilities observed. Our data suggest that genetic imprinting may be a source of this phenotypic variability. Genetic, cognitive, and electrophysiological data from 65 individuals diagnosed with WMS were analyzed with 11 polymorphic markers within the deleted region. These same subjects completed a battery of neurocognitive tests measuring general intelligence, receptive and expressive language (including measures of social and spatial components of language), visuospatial skills, and face/object processing. Finally, event-related potentials (ERP) were recorded while subjects completed a face recognition task which has been previously shown to be atypical in WMS (Mills et al., 2000). Results identified 36 maternal deletions (21 female, 15 male) and 25 paternal deletions (16 female, 9 male). Analyses uncovered a few significant differences and several trends with sample sizes ranging from 17 to 34 for a given task. The evidence suggested that individuals carrying maternal deletions obtained lower scores in the K-ABC Gestalt Closure ($p=0.019$), WAIS-R Block Design ($p=0.024$), and Benton Judgment of Line Orientation ($p=0.037$). Furthermore, trends in the same direction were also found in the DAS GCA ($p=0.091$) and WAIS-R Performance IQ ($p=0.088$). No gender or age-related differences were found between groups. Analysis of the ERP data in a face-processing paradigm revealed greater abnormality in the maternal than the paternal group, suggesting that imprinting may also play a role in the neural organization of face processing. We propose that the expression of genes in WMS may be affected by genetic imprinting, which contributes to cognitive variation in WMS and possibly in the normal population.

Analysis of candidate genes for non-syndromic craniosynostosis. *S.A.B. Boyd¹, G. Zhang¹, R. Ingersoll¹, N. Isaac¹, L. Kasch¹, D. Hur¹, E.W. Jabs¹, T. Beaty², A.F. Scott¹.* 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine; 2) Dept Epidemiology, Johns Hopkins School of Public Health.

While significant progress has been made in understanding the genetic basis of syndromic craniosynostosis, the causes of non-syndromic craniosynostosis remain to be elucidated. We have initiated a candidate gene based association study, identifying and verifying single nucleotide polymorphisms (SNPs) in a set of case-parent trios with non-syndromic craniosynostosis. The candidate genes were selected based on phenotypes caused by known mutations (i.e. syndromic craniosynostosis, animal models) and demonstrated biological function. As a first step, we performed sequencing analysis of the hot-spots for syndromic craniosynostosis: FGFR1 exon 7, FGFR2 exons 8 and 10, FGFR3 exon 7, and the entire coding sequence of TWIST. Analysis of 93 patients with non-syndromic craniosynostosis identified two FGFR3 P250R mutations in patients with coronal craniosynostosis, one of them familial. No mutations were identified in the other 91 individuals (64 sagittal, 8 coronal, 7 lambdoid, 5 metopic and 7 with multiple suture involvement). We are currently performing sequencing analysis on a group of 20 patients with isolated sagittal craniosynostosis for the entire coding sequence of the following candidate genes: FGFR1, FGFR2, FGFR3, FGFR1, SNAIL, SLUG, TWIST, and MSX2. No mutations have been identified so far in over 53 kb of genomic sequence (93 amplicons, 11.7 kb coding, 42 kb non-coding sequence) completed on 14 patients. A total of 93 SNPs [46 previously reported (as of 5/14/02) and 47 unique] have been identified. Of these, 19 SNPs occur in exons and 6 were non-synonymous (nsSNPs). The nsSNPs are being evaluated as possible disease causing variants. An additional twenty candidate genes have been selected for SNP identification. Allele frequencies will be determined and selected SNPs will be used in a large scale genotyping effort to detect evidence of linkage and disequilibrium or to identify possible mutations in families with non-syndromic craniosynostosis.

Genetic Analysis of Candidate genes in Nonsyndromic Cleft Lip Families. *L. Moreno*¹, *M. Marazita*³, *M. Arcos-Burgos*², *K. Krahn*¹, *B.S. Maher*³, *M.E. Cooper*³, *C. Valencia*⁴, *A.C. Lidral*¹. 1) Orthodontics and Dows Institute for Dental Research, University of Iowa, Iowa City, IA; 2) Department of Biology, University of Antioquia, Medellin, Colombia, SA; 3) Cleft Palate-Craniofacial Center And Department of Human Genetics, University of Pittsburgh; 4) College of Dentistry, University of Antioquia, Medellin, Colombia, SA.

Nonsyndromic cleft lip with or without palate (NSCL/P) is a common genetically, complex birth defect, with prevalence from 1/500 to 1/1000 live births. Evidence from linkage, linkage disequilibrium and mouse models studies is contradictory suggesting that heterogeneity between study populations may exist. A recent report of a genome wide scan in 92 sib pairs from the United Kingdom revealed suggestive linkage to 10 loci (Prescott et al. 2000). The purpose of this study is to replicate these results and evaluate additional candidate genes in 49 Colombian and 13 Ohio families. Genotypes were obtained for STRPs at 1p36, 2p13 (TGFA), 4p16 (MSX1), 6p23-25, 6q25-27, 8q23-24, 11p12-q13, 12q13, 14q24 (TGFB3), 16q22-24, 17q12-21 (RARA) and Xcen-q21. Linkage was performed using parametric (dominant and recessive models) and nonparametric (GENEHUNTERnpl and SimIBD) analyses. In addition, heterogeneity was analyzed using GENEHUNTER HLOD and association determined by the TDT. The Colombian families showed significant SimIBD results for 11p12-q13 ($p=0.034$), 12q13 ($p=0.015$), 16q22-24 (0.01) and 17q12-21 (0.009), while the Ohio families showed significant SimIBD results for TGFA ($p=0.005$), 6p23 ($p=0.004$) and 11p12-q13 ($p=0.025$) and a significant NPL result for 4p16 ($p=0.03$). Several families yielded LOD scores ranging from 1.09 to 1.73, for loci at 4p16, 6p23-25, 16q22-24 and 17q13. Efforts are ongoing to genotype more densely spaced markers for these loci to verify the results. In addition, candidate genes in the positive loci are being sequenced in families yielding positive results.

No association between *MTHFR* alleles and non-syndromic cleft lip/palate (nsCL/P) in three South/ Central American populations. *M.A. Sozen*¹, *M. Tolarova*², *R.A. Spritz*¹. 1) Human Medical Genetics Prog, Univ Colorado Health Sci Ctr, Denver, CO; 2) Dept Orthodontics, Univ Pacific, San Francisco, CA.

Non-syndromic cleft lip with or without cleft palate (nsCL/P) is among the most common major birth defects, occurring with a frequency of 1/500-1/2500 in most populations. The inheritance of nsCL/P is complex, with likely involvement of numerous genetic and environmental risk factors in a multifactorial interaction. Several candidate genes for nsCL/P have been studied in various human populations by linkage and association analyses, including *MTHFR*, encoding methylenetetrahydrofolate reductase, a key enzyme in folic acid metabolism. These studies have yielded conflicting results, some showing apparent allelic association between *MTHFR* polymorphisms and nsCL/P, and others finding no such association.

Recently, it has been suggested that increased maternal frequency of the *MTHFR* 665T allele, which results in reduced catalytic activity, and TT genotype in the offspring, contributes to the risk of non-syndromic cleft lip and palate. We carried out a case-control allelic association study of the two common functionally significant *MTHFR* variants, C665T and A1286C, in 408 patients with nsCL/P, 304 mothers, and 299 normal controls from three different regions of South and Central America: the Santiago del Estero region of Argentina, the Cumana region of northern Venezuela, and Guatemala. We found no consistent allelic associations for these variants between cases and controls in any of these regions. The frequency of the 665C allele was increased among mothers of nsCL/P patients in Venezuela ($p=.023$) and Argentina ($p=.052$) versus in population-matched controls, but was reduced among mothers of nsCL/P patients in Guatemala ($p=.035$). We observed no significant differences in *MTHFR* allele frequencies between nsCL/P cases versus controls. Overall, we found no consistent significant associations between these *MTHFR* alleles and nsCL/P in any of these three South / Central American populations, suggesting that these variants do not play a major role in the pathogenesis of nsCL/P in these groups.

Calpain-10 Gene Polymorphisms and Type 2 Diabetes in West Africans. *Y. Chen¹, R. Kittles¹, J. Zhou¹, G. Chen¹, K. Panguluri¹, W. Chen¹, A. Amoah², V. Opoku Acheampong², B. Eghan³, A. Nyantay³, E. Ofoegbu⁴, J. Oli⁴, F. Abbiyesuku⁵, M. Kuti⁵, T. Johnson⁶, T. Rufus⁶, H. Daniel¹, G. Dunston¹, F. Collins⁷, C. Rotimi¹ and Africa America Diabetes Mellitus (AADM) Study.* 1) National Human Genome Ctr, Howard Univ, Washington, DC; 2) University of Ghana, Accra; 3) University of Science and Technology, Ghana; 4) University of Nigeria, Enugu; 5) UCH, Ibadan, Nigeria; 6) University of Lagos, Nigeria; 7) NHGRI, NIH, Bethesda.

Recently, a number of studies in different populations reported association between genetic variations in the calpain 10 gene (CAPN10) and type 2 diabetes. In this report, we investigated whether genetic variations of CAPN10 are associated with type 2 diabetes in a cohort of siblings from four major ethnic groups in West Africa (Ghana: Akans and Ga; Nigeria: Ibos and Yorubas). Three single nucleotide polymorphisms (SNPs), SNP-43, -56 and -63 in CAPN10 gene were genotyped in 840 sibpairs and 190 controls. We observed a significant twofold increase in the risk (Odds Ratio [OR] = 2.1; 95% CI=1.12 3.77) of developing type 2 diabetes for carriers of the 221 haplotype compared to controls. The 221 haplotype is however rare in these populations with a frequency of 9% in cases and 5% in controls. All the OR associated with the haplogenotypes containing the 221 haplotype were greater than the null value of 1.0; none reached statistical significance however. We did not observe any association between type 2 diabetes and individual alleles or genotypes. In conclusion, we report a significant association between a haplotype (221) of the calpain 10 and type diabetes in our cohort of diabetes patients from West Africa. The low frequency of the at risk haplotype (221) and the lack of allelic or genotypic associations detracts from a causal interpretation.

Cladistic analysis of MAOA haplotypes and association with the risk for substance use disorders. M.M.

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Monoamine oxidase A (MAO-A) locus is an attractive candidate for exploring genetic contribution to variation in the risk for substance use disorders, because of its important role in the metabolism of neurotransmitters, including dopamine and serotonin. Our preliminary findings (Vanyukov et al., *Am J Med Genet* 60:122-126, 1995), since supported by others, have suggested an association of the MAOA gene with the risk for early onset substance use disorders (SUD). To extend this research, we genotyped four MAOA markers (two VNTR polymorphisms and two SNPs) spanning the gene to build a cladogram reflecting the evolutionary history of MAOA haplotypes (Devlin et al., in preparation). Following the ideas of Templeton et al. (*Genetics* 117:343-351, 1987), the cladogram served as the framework for nested logit and measured haplotype (nested ANOVA) analyses of association between MAOA and indices of liability to SUD (diagnosis, age of onset, and a dimensional index of substance use related problems [DUSI; Tarter, *Am J Drug Alcohol Abuse* 16:1-46, 1990]) in a sample of adult males of European ancestry. To replicate the original association finding (Vanyukov et al. 1995), we also tested in adult probands and their adolescent sons SUD liability association with a MAOA intron 2 (CA)_n repeat (STRP). Whereas no association was found in adults for the categorical diagnosis, a significant relationship was detected between the dimensional liability indices and MAOA haplotypes. Consistent with our prior results, the STRP was associated with the risk for SUD in adolescents (i.e., early onset disorder), but not in adults. Combined with our other findings reported herein (Maher B.S. et al.), these data suggest the relationship between the MAOA gene and SUD risk, possibly mediated by liability to early onset behavioral problems.

Paternal Uniparental Disomy of 11p15 is Associated with Isolated Hemihyperplasia and Expands the Beckwith-Wiedemann Syndrome Spectrum. *C. Shuman*^{1,2,3}, *L. Steele*^{2,4}, *Y.L. Fei*³, *P.N. Ray*^{2,3,4}, *E. Zackai*⁵, *M. Parisi*⁶, *J. Squire*^{7,8}, *R. Weksberg*^{1,2,3,9}. 1) Div Clin & Metabol Genetics, Hosp Sick Children, Toronto; 2) Dept Molec & Med Genetics, Univ Toronto, Toronto; 3) Research Institute, Hosp Sick Children, Toronto; 4) Dept Paediatr Lab Med, Hosp Sick Children, Toronto; 5) Div Human Genetics & Molecular Biology, Children's Hosp of Philadelphia; 6) Dept Medical Genetics, Univ Washington, Seattle; 7) Ontario Cancer Institute, Toronto; 8) Dept Lab Medicine & Pathobiology, and Dept Med Biophysics, Univ Toronto, Toronto; 9) Dept of Paediatrics, Hosp Sick Children, Toronto.

It has long been recognized that isolated hemihyperplasia (IH) is a clinically heterogeneous condition. A subset of these individuals have features in common with Beckwith-Wiedemann syndrome (BWS). These features include high birth weight, renal anomalies and embryonal tumor predisposition. Unlike BWS, however, the underlying molecular etiologies for IH has not been elucidated, with the exception of one report of a child with IH and adrenal carcinoma who subsequently developed Wilms tumor and who was found to have paternal uniparental isodisomy (UPD) for chromosome 11p. We report here paternal UPD for chromosome 11p in five patients with IH. On closer evaluation, some of these children had some features of BWS but did not meet the clinical diagnostic criteria for this disorder. Two of these five children developed hepatoblastoma; two others were macrosomic; and two had organomegaly. Thus, somatic mosaicism, which leads to abnormal expression of imprinted genes on 11p15, forms the molecular basis for IH in some individuals. UPD may be the cause of IH in other patients but may have gone undetected due to low level mosaicism, although other molecular etiologies may exist within this clinical diagnosis. The clinical findings in our five patients with a clinical spectrum including IH, together with UPD for 11p15, supports the concept of "mild" or "incomplete" BWS and expands the clinical spectrum of mosaicism for 11p15 UPD.

The HOXA1 G allele may be associated with dysmorphism in autism. *A.M. Persico¹, C. Stodgell², L. D'Agruma³, L. Zelante³, R. Militerni⁴, C. Bravaccio⁴, S. Trillo⁵, F. Montecchi⁵, C. Schneider⁶, R. Melmed⁶, M. Elia⁷, T. Pascucci⁸, S. Puglisi-Allegra⁸, K.L. Reichelt⁹, L.A. Muscarella³, V. Guarnieri³, M. Conciatori¹, P. Rodier².* 1) Mol Psychiatry & Neurogenet, UCBM, Rome, Italy; 2) OB/GYN, Univ of Rochester Sch of Med, Rochester, NY; 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-Gesù, Rome, Italy; 6) SARC, Phoenix, AZ; 7) Neurology Serv, IRCCS Oasi Maria SS, Troina (EN), Italy; 8) Dept of Psychology, Univ La Sapienza, Rome, Italy; 9) Dept of Pediatric Res, Rikshospitalet, Univ of Oslo, Norway.

The HOXA1 G allele has been associated with autism (Ingram et al 2000). This study attempts to replicate and extend these findings in 127 autistic probands from simplex Italian families, yielding 114 complete and 13 incomplete trios, and in 101 complete trios from 46 simplex and 55 multiplex Caucasian-American families. The A, and not the G allele, displays a significantly higher frequency in 127 Italian autistics contrasted with 173 ethnically-matched population controls ($\chi^2=6.41$ and 3.87 for genotypes and alleles, respectively, 1df, $P<0.05$). Transmission of the A allele from heterozygote parents to affected offspring is significantly favored over the G allele in 215 complete trios (TDT $\chi^2=6.05$, 1df, $P<0.05$), especially in simplex, but not in multiplex families, and to a larger extent in Italian than in Caucasian-American trios. Several individuals carrying one- or three-histidine deletions were also identified. Histidine deletions were consistently found in A/G individuals, more frequently on A than on G alleles, and were not associated with autism. Since 32/57 (56.1%) autistic probands assessed by Ingram et al (2000) displayed mild-to-moderate dysmorphic features, a cause for exclusion from the current study, we hypothesize that the HOXA1 G allele may be associated with dysmorphic features seen in autism-spectrum disorders. Supported by Telethon-Italy (E.0858), Italian Min. Health (ICS.190.1.RF99/90), CNR (99.00555.PF33), and N.A.A.R.

Liver pyruvate kinase polymorphisms are associated with type 2 diabetes in Northern European Caucasians. *S.K. Das¹, H. Wang¹, Q. Ren¹, W. Chu¹, S.J. Hasstedt³, S.C. Elbein^{1,2}.* 1) Medicine, University of Arkansas for Medical Sciences, Little Rock, AR; 2) Central Arkansas Veterans Healthcare System, Little Rock, AR; 3) Department of Human Genetics, University of Utah Health Sciences Center, Salt Lake City, UT.

Pyruvate kinase is a key glycolytic enzyme. Isoforms that are expressed in the red cell, liver, pancreatic β cells, small intestine, and proximal renal tubule are encoded by the 12 exons of the PKLR gene, which maps to chromosome 1q23. We hypothesized that common variants of the PKLR gene could account for the linkage of diabetes to this region. We screened the promoter regions, exons and surrounding introns, and the 3' untranslated region for mutations. We identified 5 single nucleotide polymorphisms (SNPs); only one (V506I, exon 11) altered the coding sequence. We tested the 5 SNPs, a poly-T insertion-deletion polymorphism, and an ATT triplet repeat in 131 unrelated diabetic individuals and 118 non-diabetic control individuals. The V506I variant was rare and not associated with type 2 diabetes. The 4 SNPs and the insertion deletion polymorphism were associated with diabetes with a 10% difference in allele frequency between individuals with diabetes and nondiabetic individuals (p 0.001 to 0.011; relative risk for minor allele 1.85). The same trend was found for the ATT repeat ($p=0.029$). The 5 diallelic variants, spanning 9.4 kb, were in strong linkage disequilibrium. Thus, 98.4% of all haplotypes fell into two classes. We next determined these two haplotypes in 698 individuals (283 affected) from 63 kindreds. No excess transmission to affected offspring was found, and neither haplotype was associated with glucose or insulin levels, nor with measures of insulin secretion. Common variants in the PKLR are associated with increased risk of type 2 diabetes, but because of strong linkage disequilibrium between variants, the actual susceptibility allele may be in a different gene. Additional studies are required to reconcile the lack of association on transmission disequilibrium testing and the lack of effect on measured phenotypes with apparent the population association.

A role of hereditary hemochromatosis HFE gene mutations in susceptibility to type 2 diabetes mellitus in a Polish population. *M.T. Malecki¹, T. Klupa¹, M. Walus¹, W. Czogala¹, J. Bochenski², P. Greenlaw¹, J. Sieradzki¹.* 1) Dept. of Metabolic Diseases, Jagiellonian University, Krakow, Poland; 2) Joslin Diabetes Center, Boston, MA, USA.

Hereditary hemochromatosis (HH) is a frequent, autosomal recessive metabolic disorder characterised by excess iron deposition in various organs. Two mutations in the HFE gene are associated with HH. Due to the high prevalence of type 2 diabetes mellitus (T2DM) in HH, a hypothesis exists that heterozygous carriers of HFE mutations may be at a higher risk of developing T2DM. Aims: 1) To identify the frequency of C282Y and H63D mutations in a population of from the Malopolska region in southeastern Poland; 2) To search for an association of HFE mutations with T2DM in this population. Methods: We included 391 individuals in this study: 222 T2DM patients and 169 controls. The fragments of HFE were amplified by PCR. Alleles and genotypes were determined by gel electrophoresis of the DNA digestion products from SnaBI and DpnII, respectively. Differences in distributions were examined by χ^2 test. Results: At codon 282 the frequency of C/Y alleles was 98.2%/1.8% in T2DM patients and 96.7%/3.2% in controls ($p=0.19$). At codon 63 the frequency of H/D alleles were 85.6%/14.4% and 88.8%/11.2% ($p=0.19$), respectively. The distribution of genotypes was not statistically different between the two groups, although a trend of higher frequency of HD and DD genotypes was observed in T2DM group (27.4% vs. 20.1%, $p=0.09$). However, in the stratified analyses based on T2DM age of onset and gender, we observed a higher prevalence of HD and DD genotypes among T2DM patients diagnosed at > 49 years of age, the mean age for the entire group, ($p=0.018$) and among male T2DM individuals ($p=0.005$) than in the controls. Conclusion: 1) The frequency of HH associated mutations in this population from southeastern Poland is similar to other Caucasians; 2) We did not find evidence for the recently reported association of C282Y mutation with T2DM in a Polish population. However, the results of our study suggest that the H63D mutation may play a role in the pathogenesis of late onset T2DM and in males in a Polish population.

Mutation scan of the human Interleukin-18 Binding Protein: A candidate gene for type 1 (insulin dependent) diabetes. *R. Nolsoe¹, F. Pociot¹, D. Novick², M. Rubinstein², S.-H. Kim², C.A. Dinarello³, T. Mandrup-Poulsen¹.* 1) Steno Diabetes Ctr, Gentofte, Denmark; 2) Weizmann Institute, Rehovot, Israel; 3) University of Colorado Health Sciences Ctr, Denver, CO.

Background/Aims. Type 1 (insulin dependent) diabetes, T1D, is the result of an immune mediated destruction of the pancreatic beta cells dependent mainly on T helper cells and macrophages. Interleukin-18, IL-18, is a proinflammatory cytokine produced mainly by macrophages. IL-18 is capable of inducing T lymphocyte synthesis of IFN γ thereby skewing the T helper response toward a T helper type 1 profile. Interleukin-18 binding protein, IL18BP, neutralizes interleukin 18 and leads to a reduced T helper type 1 response. Polymorphisms in *IL18BP* may affect IL18 activity and the magnitude of the T1 helper response and may play a role in the pathogenesis of T1D. The aim of the study was therefore to identify polymorphisms in *IL18BP* and to test these for association to T1D. **Methods.** We evaluated the human IL18BP gene on chromosome 11q13 as a candidate susceptibility gene for T1D and scanned the entire *IL18BP* (promoter, exons 1-4 and 3'UTR) for polymorphisms using single strand conformational polymorphism analysis and direct sequencing. **Results.** We identified a total of eleven polymorphisms, all having allele frequencies ranging between 0.05 and 0.10. Four were in the promoter region; -257G>T, -78C>T, -65G>A and -59Gins. Three were in intron 2; 1869A>C, 2226G>T and 2314G>T and the last four; 3071A>T, 3081T>A, 3421C>G and 3474Gins were in the 3'UTR of *IL18BP*. **Conclusion.** We identified eleven polymorphisms in *IL18BP*. However, none of these were frequent enough to permit association studies in T1D, and we conclude that *IL18BP* does not contribute to the overall genetic susceptibility to type 1 diabetes.

A high resolution SNP association scan for IDDM1. *M. Wjst*¹, *N. Herbon*¹, *M. Werner*¹, *E. Bonifacio*², *A. Ziegler*². 1) Inst fuer Epidemiologie, GSF Forsch Umwelt & Gesundh, Munich, Germany; 2) Diabetes Research Institute, Munich, Germany.

Type 1 diabetes (T1D) is an autoimmune disease which is highly associated with HLA class II genes in the major histocompatibility complex (MHC) on chromosome 6p21. Association analysis of the MHC region reveal certain highly predisposing or protective haplotypes designed as IDDM1. Although the contribution of additional susceptible loci with minor effects is likely they could not be identified because of strong linkage disequilibrium in this region.

We tested in a case-control approach study 534 SNPs selected from an initial set of 1,435 SNPs covering 25 Mb around the HLA complex. We compared the allele frequencies between DNA pools of 192 German late onset T1D patients and 288 healthy individuals from a South German population-based survey using MALDI-TOF.

The already known association with the haplotypes DR3 and DR4 could be confirmed by highly significant allele differences of four SNPs located between DQB1 and DRB1 genes. Additionally, association of T1D with DOB and TNF as risk modifiers could be reproduced. We found three further potential disease loci in the HLA-class I respectively class III region which remain to be confirmed in individual haplotypes.

These findings suggest that allele frequency analysis of randomly selected markers even with low polymorphism information content is a promising method to identify disease associated loci in large linkage regions.

Association Study of *Fas* and *FasL* Polymorphisms with Multiple Sclerosis. *B.G. Weinshenker*¹, *D.D. Hebrink*¹, *O.H. Kantarci*¹, *S.J. Achenbach*², *E.J. Atkinson*², *C.T. McMurray*³. 1) Department of Neurology, Mayo Clinic and Foundation, Rochester, MN; 2) Department of Health Sciences Research, Mayo Clinic and Foundation, Rochester, MN; 3) Departments of Pharmacology, Biochemistry and Molecular Biology, Mayo Clinic and Foundation, Rochester, MN.

Fas and *FasL* expression leads to antigen activation-induced apoptosis of T-cells. Mutations in *Fas* and *FasL* cause autoimmunity and lymphoproliferation in mice and humans. Others have reported association of polymorphisms adjacent to or in *Fas* and *FasL* with multiple sclerosis (MS) susceptibility. We studied the association of *Fas* and *FasL* polymorphisms with susceptibility to, gender bias, age at onset, course and disability indexed to time of MS. Three SNPs per gene were selected to establish haplotypes that span the genes. We genotyped a population-based sample of 122 cases and 244 gender, age and ethnicity-matched controls using PCR-RFLP. Results were not corrected for multiple comparisons in this preliminary analysis. There was linkage disequilibrium (LD) between 5'(-670) and Exon7(74) SNPs of *Fas* ($p < 0.00001$). MS was more frequent in women, but not in men, who were homozygous for 5'(-670)*A ($p = 0.034$; OR:1.78, 95% CI:1.04-3.03) and for Exon7(74)*C ($p = 0.019$; OR:1.84, 95% CI:1.10-3.07), the specific alleles in LD, and for the haplotype defined by these alleles ($p = 0.053$; OR:1.66, 95% CI:0.99-2.78). Homozygotes for Exon7(74)*C more frequently had a primary progressive versus bout onset course ($p = 0.057$; OR:3.92, 95% CI:0.88-14.43). None of the *Fas* polymorphisms was associated with disease severity or age at onset. There was LD between 5'(-663), Intron2(1153) and Intron2(2776) SNPs of *FasL* ($p < 0.00001$). Carriers of 5'(-663)*T had increased susceptibility to MS ($p = 0.005$; OR:2.02, 95% CI:1.23-3.32), the effect being primarily due to a difference in the frequency of heterozygotes. None of the *FasL* polymorphisms was associated with age of onset, severity or course.

Two SNPs of *Fas* in LD with one another and a 5' region SNP of *FasL* may be associated with susceptibility to MS. For the *Fas* SNPs, the effect was evident only in homozygous women.

Cladistic analysis of MAOA haplotypes and association with the risk for attention deficit hyperactivity disorder.

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The monoamine oxidases (MAO-A and MAO-B) are of interest in the study of many behavioral traits and disorders because of their role in the nervous system. Prior findings suggested contribution of the MAOA gene to variation in impulsivity, aggression and the risk for substance use disorder (SUD). SUD is frequently preceded by attention deficit hyperactivity disorder (ADHD). In turn, the highly heritable dimensional traits of hyperactivity-impulsivity and inattention underlie the risk for ADHD. We hypothesized that hyperactivity-impulsivity is associated with the MAOA gene, possibly mediating the MAOA association with the risk for ADHD. As part of an ongoing study, we inferred a cladogram representing the evolutionary relationship of MAOA haplotypes based on four markers (two VNTR polymorphisms and two SNPs) spanning the gene. Based on this cladogram, we tested for association between MAOA haplotypes and ADHD-related traits (hyperactivity-impulsivity and inattention), using measured haplotype analysis. We also tested for association between an STRP in intron 2 of MAOA ([CA]_n repeat), previously shown to be related to the risk for SUD, and the ADHD-related traits. ADHD was quantified in 163 10-12 year old Caucasian males using DSM-III-R criteria. Hyperactivity-impulsivity and inattention scores used in the study were derived via factor analysis. No association was found between the haplotypes and ADHD risk dimensions. However, moderate association (without Bonferroni correction) was detected between the MAO STRP and hyperactivity-impulsivity ($p=0.02$). This finding supports the hypothesis that variants in MAOA affect liability to ADHD, albeit weakly. Further research into this apparent association is required.

Association analysis guided by the evolution of haplotypes. *H. Seltman*¹, *K. Roeder*¹, *B. Devlin*². 1) Department of Statistics, Carnegie Mellon University, Pittsburgh, PA., USA; 2) Department of Psychiatry, University of Pittsburgh School of Medicine.

Association studies, both family-based and population-based, are a powerful means of detecting disease-liability alleles. To increase the information of the test, various researchers have proposed targeting haplotypes. Haplotypes indeed produce more definitive transmissions than the alleles comprising them, and this tends to increase power. However, the larger number of haplotypes, relative to alleles at individual loci, tends to decrease power because of the additional degrees of freedom required for the test. An optimal strategy would focus the test on particular haplotypes or groups of haplotypes much as has been done with measured haplotype analysis (MHA). First suggested by Templeton and colleagues, MHA uses the evolutionary relationships among haplotypes to produce a limited set of hypothesis tests and to increase the interpretability of these tests. To fully utilize the information contained in the evolutionary relationships among haplotypes, we propose a number of methodological extensions for the analysis of data from family-based and population-based studies. These extensions account for haplotype phase ambiguity; detect likely recombinants; incorporate covariates as needed; model correlation among siblings when they are available; and can detect likely errors in genotyping and paternity assignment. While not all of the methods are new, they are integrated into a software package that allows for exploratory data analysis and tests for association in samples of multilocus genotypes around candidate genes.

Triplet repeat sizes in candidate genes for Spinocerebellar Ataxia. *C. Homem¹, P. Magalhães¹, J. Sequeiros^{1,2}, I. Silveira¹*. 1) UnIGENE, IBMC, Porto, Portugal; 2) Laboratório Genética Médica, ICBAS; University of Porto, Portugal.

Spinocerebellar ataxias (SCAs) are a heterogeneous group of neurodegenerative disorders with a large range of clinical manifestations. At least fifteen genes are implicated in autosomal dominant SCAs: SCA1, SCA2, SCA3/MJD, SCA4, SCA5, SCA6, SCA7, SCA8, SCA10, SCA11, SCA12, SCA13, SCA14, SCA17 and the dentatorubropallidoluysian atrophy (DRPLA) gene. A triplet repeat expansion is responsible for 10 of these types of SCAs, but there are still many affected families not assigned to any of them. To identify additional genes implicated in these disorders, we selected nine expressed brain genes with triplet repeat tracts, which thus are good functional candidates for these neurodegenerative diseases. Repeat sizes were assessed for CALM1, MAB21L1, STC1, CBP, NUMBL, GRIK2, KCNN3, RAI1 and Clone 2.70 in 73 unrelated patients with SCA. All these patients were previously excluded for SCA1, SCA2, SCA3/MJD, SCA6, SCA7, SCA8, SCA12, SCA17 and DRPLA. Two genes that account for a significant number of expansions found by RED analysis were also studied, ERDA1 and SEF2-1. Large alleles were not found at any of the nine candidate genes. The ERDA1 gene showed CAG/CTG alleles of 50 to 81 repeats in 20 patients, including two affecteds from one SCA family with dementia and chorea associated. A large allele with 86 CTGs was also found at the SEF2-1 gene. In conclusion, the functional candidate genes selected do not seem to be responsible for SCA in our families.

Regions of Instability and Mutability at the *SCA8* locus. *S. Martins*¹, *P. Magalhães*¹, *I. Alonso*^{1,2}, *J. Sequeiros*^{1,2}, *I. Silveira*¹. 1) UnIGENE, IBMC, Porto, Portugal; 2) Laboratório Genética Médica, ICBAS, Universidade do Porto, Portugal.

Spinocerebellar ataxia type 8 (SCA8) is a form of late-onset neurodegenerative disorder characterized by gait, limb and speech incoordination, in addition to other variable signs. An untranslated (CTG)_n expansion on chromosome 13q21, preceded by a polymorphic stable CTA tract, has been described as being responsible for this disease. However, its pathogenic role remains controversial as very large alleles have been observed, not only in SCA8 patients, but also in patients with psychiatric disorders and in healthy control subjects. Therefore, reduced penetrance, as well as genetic instability and a maternal bias towards expansion are still a matter of debate. We have previously reported a high instability in spermatogenesis of both expanded and normal alleles, suggesting a high mutational rate at the *SCA8* locus. In order to gain insight into the biological role of the *SCA8* locus, we 1) performed a haplotype analysis, including the CTA-CTG polymorphism and eight SNPs flanking the repeat, and 2) looked for intergenerational instability and mutability in this region, in five SCA8 families and 20 control families. The analysis of two exonic SNPs (a A/G and a T/del) and an intronic C/T, upstream from the repeat, showed a common haplotype associated to the disease in all SCA8 families. In the control families, the most frequent allele had 24 CTA-CTG repeats. Interestingly all the possible haplotypes resulting from the A/G and C/T polymorphisms were seen with this allele, which suggests a higher instability rate for the 24 CTA-CTG repeat. We are completing haplotypes with the additional SNPs to identify the entire region shared by our SCA8 patients, as well as the mutability region.

A rigorous test of the CAG trinucleotide repeat expansion hypothesis of schizophrenia. *T. Tsutsumi¹, S.E. Holmes¹, A. Sawa¹, C. Callahan¹, M.G. McInnis¹, C.A. Ross¹, L. DeLisi², R.L. Margolis¹.* 1) Dept of Psychiatry, Johns Hopkins University, Baltimore, MD; 2) Dept of Psychiatry, New York University, New York, USA.

We and others have proposed that the genetic risk for schizophrenia and other psychiatric disorders could at least partly stem from expansion of trinucleotide repeats, particularly of the CAG/CTG type. To search for novel CAG/CTG repeat expansions, we examined 100 unrelated probands with familial schizophrenia using the Repeat Expansion Detection (RED) technique, an assay that detects the presence of CAG/CTG repeats larger than about 50 triplets in length in genomic DNA. 28 subjects with CAG/CTG expansions were detected using the RED assay. Using PCR and genomic Southern blots, we tested each RED positive sample for all known pathogenic and nonpathogenic repeat expansions. The expansion in 20 subjects could be accounted for by the nonpathogenic expansion at the Dir1 locus on 17q21. 7 of the 8 remaining subjects had expansions of the CTG repeat in SEF2-1 on 18q21, another expansion with no known consequences. The remaining subject had an expansion of the spinocerebellar ataxia type 8 (SCA8) locus on 13q21; this expansion may cause spinocerebellar ataxia type 8, but also occurs in the normal population. In conclusion, all RED positive cases could be explained by expansion of one of three loci known to undergo CAG/CTG repeat expansion in the normal population. While none of the repeat expansions that we detected were novel, we cannot exclude the possibility that the SEF2-1 repeat, expanded in 7% of our patients vs. 3% of the general population, could contribute to the risk for schizophrenia. This analysis also does not address CAG/CTG expansions below the threshold of detection for RED or other types of repeats, several of which have been associated with CNS disease.

Bipolar and Panic Disorder: Common and Syndrome-Specific Genes? *M. Durner*¹, *AJ. Fyer*², *SE. Hodge*^{1,2}, *SP. Hamilton*², *JA. Knowles*^{2,3}, *MM. Weissman*^{2, 4}. 1) Biostatistics; 2) Psychiatry; 3) Genome Center; 4) Epidemiology, Columbia University, New York.

A high comorbidity of bipolar disease (BP) and panic disorder (PD) has been noted in epidemiological studies. Additionally, genetic studies in BP have indicated also a genetic connection between BP and PD. We hypothesize that there may be two types of genes for BP and PD - some genes shared by both diseases, but also syndrome specific genes and it will be the interaction of both that will produce the particular phenotype. We tested this hypothesis in a dataset of 120 multiplex PD families, of whom 37 also had ≥ 1 member affected with BP. A complete genome scan was performed in all 120 families by CIDR. To search for a common gene for BP and PD, we first analyzed all families together, treating any family member with either PD or BP as affected. Secondly we analyzed families separately, depending on whether they had at least one BP member (PD+BP) or none (PD-BP). We calculated 2-point lodscores, allowing for independent male and female recombination fractions $q = (q_m, q_f)$, and assuming dominant (DOM) and recessive (REC) inheritance with an arbitrary penetrance of 50%. Search for common genes: The highest maxlod (Z_{\max}) of 4.9 at $q = .1, .3$ occurred at D2S125 (under DOM). When we considered only PD, but not BP as affected, the lod dropped to 4.5. Another peak occurred on chr.12. At PAH, Z_{\max} was 4.3 ($q = .1, .3$). Assuming REC, we found a $Z_{\max} = 4.3$ ($q = .1, .01$) at D17S1298. . Both PD-BP and PD+BP families contributed proportionally to these lods indicating that these loci are involved in both types of families. Search for syndrome specific genes: Analyzing PD+BP and PD-BP families separately, we found evidence for linkage in PD-BP families at D4S1644 with $Z_{\max} = 3.1$ and at D5S211 with $Z_{\max} = 3.3$. The PD+BP families had negative lods at these locations. The Predivided-Sample Test showed a significant difference between these two groups ($p < 0.5$). In PD+BP families, Z_{\max} was 3.0 at D13S779, vs. 0.9 in PD-BP families. Our findings might indicate genes common for PD and BP on chr.2, 12 and 17 interacting with genes on chr.4, 5 or 13 to produce the bipolar or panic phenotype.

Genetic Contribution to Uric acid in hypertension families : The Korean Cardiovascular Genome Study. *E.Y*

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Hyperuricemia is predictive for the development of hypertension and is associated with increased cardiovascular morbidity and mortality. Serum uric acid level is affected by multiple factors which is known to be a coronary artery risk factor and genetic factors. To address this issue, we investigated environmental, familial, and genetic influences on uric acid in 635 members of 136 families who had participated in the Korean Cardiovascular Proband Study. Proband was diagnosed essential hypertension and had not nephropathy. Maximum likelihood methods were used to fit several genetic and nongenetic models of inheritance. Adjustments for age, BMI, blood pressure, lipid levels, smoking and drink status were carried out separately for males and females by multiple regression procedures for uric acid phenotypes prior to segregation analysis. Uric acid was normally distributed and ranged from 2.00 to 11.00, with a mean of 4.86 (male, 5.68 and females, 4.09) in the entire samples. Serum uric acid levels had positive correlation with BMI, systolic blood pressure in males and with age, BMI in females. Adjusted uric acid showed weak spouse correlation (0.27) and showed stronger parent-offspring correlation and sibling correlation (0.32, 0.45) respectively. The most parsimonious model was a dominant model and estimated heritability of the uric acid was 0.681. Our results showed the major gene effect determining the uric acid in the Korean population, and linkage analyses using the genetic markers should be followed for identification of candidate genes.

ANOTHER LOCUS FOR AUTOSOMAL DOMINANT LATE ONSET ALZHEIMERS DISEASE IN BRAZILIAN FAMILIES ? *A.L. Nishimura¹, J.R.M. Oliveira², P.R. Brito-Marques³, R. Nitrini⁴, P.H. Bertolucci⁵, I.H. Okamoto⁵, M. Zatz¹.* 1) Dept. Biologia, Universidade de So Paulo, São Paulo, São Paulo, Brasil; 2) Department of Neurology, UCLA, USA; 3) Centro de Neurologia Cognitiva e do Comportamento da Universidade de Pernambuco, Pernambuco, Brasil; 4) Departamento de Neurologia da Faculdade de Medicina, USP, Brasil; 5) Departamento de Neurologia e Neurocirurgia da UNIFESP, Brasil.

Alzheimers disease(AD), the most common form of dementia in elderly, is characterized by a progressive deterioration in memory, language and others cognitive functions. Three autosomal dominant genes (the Amyloid Precursor Protein - APP, Presenilin 1 - PSEN1 and Presenilin 2 - PSEN2) responsible for early onset familial AD (AD-FAD) have been identified. However, familial AD accounts for less than 1% of all cases. Among the several susceptibility loci responsible for late onset AD (LOAD), the APOEε4 allele is the most important risk factor worldwide. Moreover, a functional polymorphism on the serotonin transporter promoter (5-HTTLPR) has been reported as a susceptibility gene to AD. We have ascertained four families with patients affected by AD displaying an autosomal dominant pattern of inheritance. A total of 10 patients were personally examined in these families and the diagnosis criteria was based on NINCDS-ADRDA. Linkage analysis allowed to exclude the autosomal dominant APP, PSEN1, PSEN2 genes as responsible for AD in three of these families. In addition, the association with the Apoe4 allele or the 5-HTTLPR polymorphic locus and AD was not found in all patients, although these markers represent only susceptibility loci. Based on these results, we suggest the existence of another gene, or more genes for autosomal dominant Alzheimer Disease in Brazilian patients. Supported by FAPESP, PRONEX and CNPq.

Familial Parkinsonism in an Ohio Amish Pedigree. *S.L. Lee^{1,2}, A. Crunk², L. McFarland², Y. Bradford², T.L. Davis¹, J.L. Haines²*. 1) Department of Neurology, Vanderbilt University Medical Center, Nashville, TN; 2) Program in Human Genetics, Department of Molecular Physiology and Biophysics, Vanderbilt University Medical Center, Nashville, TN.

Genetic analysis in rare familial cases of Parkinson disease (PD) have identified three genes, alpha-synuclein, parkin, ubiquitin carboxy-terminal hydrolase-L1, and several chromosomal regions as being involved in PD. The discovery of additional hereditary causes may further elucidate the underlying pathogenesis and provide new pharmacological targets. From 1998 to 2002, individuals from an Amish community near Holmes County Ohio were interviewed and examined for parkinsonism and/or dementia. Fifteen affected individuals from 13 nuclear families were evaluated and identified as having PD. Genealogical research revealed a common ancestor through seven generations, generating a very large and complex single pedigree. The age range of affected individuals is 46 to 85 years. The average age of onset is 53 years with a range of 39 to 74 years. Clinical characteristics were typical for idiopathic PD, including rest tremor of asymmetric onset, bradykinesia and rigidity without early postural instability. Those who were treated with levodopa were responsive to medications. In addition, there was one family member who appeared to have essential tremor, and another with progressive supranuclear palsy. Initial genetic analysis with markers in existing regions suggest that this family has a novel gene. A genomic screen with approximately 350 microsatellite markers is underway. Preliminary analyses suggest possible locations on chromosomes 5 and 3.

Absence of linkage to the chromosome 4q24 Finnish migraine locus among 78 migraine families of European descent. *T. Wieser*¹, *J. Pascual*², *M. Barmada*³, *M. Soso*⁴, *A. Oterino*², *K. Gardner*⁴. 1) Martin-Luther-University Halle, Dept. of Neurology, Germany; 2) University Marques de Valdecilla, Dept. of Neurology, Spain; 3) University of Pittsburgh, Dept. of Human Genetics, USA; 4) University of Pittsburgh, Dept. of Neurology, USA.

Objective: Investigate 78 migraine families for linkage to the Finnish migraine with aura locus on Chromosome 4q24. **Background:** Migraine with and without aura is thought to be a genetically complex disorder. A genome scan for familial migraine with aura recently found a single locus of significance (LOD 4.2) on 4q24 among 50 Finnish families. Significant migraine loci had previously been found only for rare highly penetrant autosomal dominant hemiplegic migraine families. Replication of linkage data in broader ethnic groups is necessary to establish the importance of a locus identified in a genetic isolate. **Methods:** We ascertained 78 families (average size 7 member/family) with probands identified in headache clinics using International Headache Society criteria for headache type and genotyped markers D4S1560, D4S1647 and D4S250 using a 3700 ABI PRISM DNA Analyser. Alleles were assigned using the GeneScan Analysis software. Analysis for affected relative allele sharing and linkage was performed using ALLEGRO, VITESSE 1.1 programs and the maximized maximum LOD score (MMLS) method using several models. **Results:** Seventy-eight multi-generational families were ascertained from North America (33), Spain (27), and Germany (18). 135 individuals had migraine without aura (35 families), 74 migraine with aura (25 families), 68 hemiplegic migraine (18 families), 6 of other type (mixed 5, acephalgic migraine 1) and 79 unknown (not fulfilling diagnostic criteria). More than 50% of affected individuals and 55% of families had migraine aura (including 23% hemiplegic) compared with the Finnish families. The combined two point as well as multipoint LOD scores were below 2 for all three markers. **Conclusion:** Exclusion of the chromosome 4q24 migraine aura locus among 78 families of European descent suggests the finding may be restricted to the Finnish population and correlates with expected genetic heterogeneity.

SCREENING OF SPINK1 MUTATIONS INDICATE FURTHER HETEROGENEITY FOR CHRONIC

PANCREATITIS. *A.L. Bernardino¹, D.R. Guarita², C.B. Mott², M.R.A. Pedroso², M.C.C. Machado², A.A. Laudanna², C.M. Tani², F.L. Almeida¹, M. Zatz¹.* 1) Centro de Estudos do Genoma Humano, Dept Biologia, Universidade de São Paulo, São Paulo, Brazil; 2) Setor de Gastroenterologia, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brasil.

Chronic Pancreatitis is defined as a continuing inflammatory disease of the pancreas, characterized by irreversible morphological change and/or permanent impairment of function. Recent studies have indicated that mutations in the serine protease inhibitor, Kazal type 1 (SPINK1) gene are associated with chronic pancreatitis. However, the significance and inheritance pattern of SPINK1 mutations has been differently interpreted by different groups. We have screened 87 Brazilian unrelated patients with chronic pancreatitis (alcohol-related disease in 64, idiopathic disease in 16 and hereditary disease in 2) and 5 patients with acute idiopathic pancreatitis for mutations in the SPINK1 gene and also for the CFTR and PRSS1 genes. Five alterations were identified in the SPINK1 gene (-253T>C, -164G>C, 1-7T>G, c75C>T, +32C>T). It is important to emphasize that the missense mutation N34S, which has been associated with chronic pancreatitis among Caucasians, was not found in our patients. The same samples were tested in another study with CFTR and PRSS1 genes. Mutations in the CFTR gene were found in 8 patients (~9%) with chronic pancreatitis. In the PRSS1 gene only the E79K change was found in a patient with alcohol-related chronic pancreatitis. These results, in Brazilian patients, reinforce the importance of the molecular analysis of the CFTR gene in chronic pancreatitis. Moreover our study indicates the existence of at least one more gene responsible for hereditary and idiopathic chronic pancreatitis. Supported by FAPESP, PRONEX, CNPq.

Sequence variation in the CARD15 (NOD2) gene and susceptibility to Crohn's disease. *K. KING, M.M. MIRZA, S.A. FISHER, A.P. CUTHBERT, C.M. LEWIS, C.G. MATHEW.* Div of Med and Mol Genetics, GKT School of Medicine, LONDON, UK.

Crohn's disease (CD), a subtype of inflammatory bowel disease (IBD), is a complex disorder, with both genetic and environmental aetiology. Three sequence variants (R702W, G908R and 1007fs) in the CARD15 gene have been shown to be associated with susceptibility to CD. Genotype relative risks (GRRs) for these disease susceptibility alleles (DSAs) in our cohort of 688 unrelated cases of CD were 3.0 for heterozygotes and 24 for homozygotes/compound heterozygotes. There is also evidence of an excess of rare CARD15 variants in CD, but whether these are true DSAs is unknown. In order to investigate the contribution of rare variants to CD we have selected a panel of 192 British CD patients who are heterozygous for either R702W, G908R or 1007fs for comprehensive mutation screening. The 12 exons and 5' and 3' UTRs of CARD15 were screened for mutations using the WAVE® System, and amplicons containing heteroduplexes sequenced to identify both common polymorphisms and rare potential DSAs. Screening of the first panel of 31 heterozygous CD patients revealed four rare variants. The parents of these patients were genotyped to determine whether the rare variant was cis or trans to their known DSA. Three of the four rare variants (A612T, N852S, V955I) were in trans i.e. inherited from the parent who did not carry the known DSA, and one (M863V) was transmitted on the same haplotype as 1007fs. All four rare variants are located in sequence that encodes the functional leucine-rich repeat region of CARD15. Rare variants that are candidate DSAs are being genotyped using the TaqMan allelic discrimination assay in a cohort of 1000 CD cases and 1000 controls to determine whether they have a significantly elevated frequency in CD. The results of this study may clarify whether the genetic model for CARD15 mutations best fits: (i) a gene dosage model with a lower risk for heterozygotes than homozygotes or compound heterozygotes, or (ii) a fully recessive model with no increased risk for heterozygotes and high genotype relative risk in homozygotes.

Identification and characterization of *BBS2L1*, a paralog of the Bardet-Biedl syndrome gene 2. *J.L. Badano*^{1,3}, *S.J. Ansley*¹, *R.A. Lewis*^{3,4,5,6,7}, *J.R. Lupski*^{3,5,7}, *N. Katsanis*^{1,2}. 1) Institute of Genetic Medicine, Johns Hopkins University; 2) Wilmer Eye Institute, Baltimore, MD; 3) Departments of Molecular and Human Genetics; 4) Ophthalmology; 5) Pediatrics; 6) Medicine; 7) The Texas Children's Hospital, Baylor College of Medicine, Houston, TX.

Bardet-Biedl Syndrome (BBS) is a genetically heterogeneous disease characterized by obesity, retinal dystrophy, polydactyly, hypogenitalism, learning difficulties and renal malformations. Based on an autosomal recessive mode of inheritance six loci have been mapped (*BBS1-6*), three of which have been cloned and shown to cause disease independently and in various combinations. While the predicted protein sequence of *BBS4* and *BBS6* have provided some clues about their biological role, the amino acid sequence of *BBS2* does not reveal any biological information as it does not exhibit any similarity to genes of known function nor does it contain any recognizable motifs, with the exception of a coiled-coil domain at the amino terminus of the protein. Since the orthologs of *BBS2* exhibit substantial conservation across the entire protein, we sought to identify non-orthologous members of the *BBS2* family in humans and other organisms and thereby identify regions of *BBS2* with elevated evolutionary conservation that likely represent novel domains. We report the cloning and characterization of a novel gene in human and mouse, *BBS2L1* (*BBS2* Like 1). *BBS2L1* maps to chromosome 4q23 and encodes a predicted protein of 672 amino acids. *BBS2* and *BBS2L1* show 37% similarity and 26% identity over a specific 260 amino-acid region, which encodes a putative six-blade propeller structure with similarity to the integrin protein family. This represents the first recognizable *BBS2* domain and is likely to be important in the elucidation of the cellular role(s) of this protein family. Although mutation analysis of *BBS2L1* on a cohort of 80 BBS families did not reveal any clear pathogenic alterations, the possibility that *BBS2L1* shares some functional role with *BBS2* cannot be excluded.

KCNAB1 is not responsible for predisposition to a subgroup of juvenile myoclonic epilepsy. *O.V. Evgrafov¹, F.L. Zheng^{1,2}, P. Tabares^{1,2}, M. Durner², D. Pal², C. Gilliam¹, D.A. Greenberg^{1,2}.* 1) Genome Center, Columbia University; 2) Div of Statistical Genetics, Columbia University, NY NY.

Idiopathic generalized epilepsy (IGE) is a set of syndromes with little if any involvement of environmental factors, suggesting a completely genetic, and likely oligogenic, origin. Several gene locations have been suggested in different studies, one of which is on chromosome 3 (Sander et al, 2000). Interestingly, in our data, we found evidence for linkage in a subgroup of IGE in that area of chr. 3: juvenile myoclonic epilepsy (JME) without absence in the family. The multipoint lodscore maximized around D3S1614 (178 cM) with a value of 2.3. There is an obvious candidate gene in the region, KCNAB1, which codes for a beta 1 subunit of the potassium voltage gated channel. This gene is expressed in brain and the corresponding protein modulates gating properties of the channel. It is a suggested counterpart of the alpha subunit coded by the KCNA5 gene. Some of the known genes responsible for rare mendelian epilepsies are potassium channels. We have sequenced the coding and promoter regions of KCNAB1 in probands from 5 families with the strongest linkage evidence in this region. We detected no mutation in KCNAB1, minimizing the probability that the gene is involved in etiology of JME. [Support: NIH NS27941, DK31775, MH48858].

Primary Open-Angle Glaucoma (POAG) Candidate Gene Analysis. *K.R. LaRocque¹, M.A. Hauser¹, J.L. Wiggs², B. Broomer¹, E.A. del Bono², F.L. Graham¹, M.A. Pericak-Vance¹, J.L. Haines³, R.R. Allingham¹.* 1) Duke Univ Medical Ctr, Durham, NC; 2) Massachusetts Eye and Ear Infirmary, Boston, MA; 3) Vanderbilt University, Nashville, TN.

We have previously reported linkage analysis of 86 POAG pedigrees to identify the chromosomal locations of POAG susceptibility genes (Wiggs et al, *Hum. Mol. Genet.*, 9(7); 1109-1117). For these studies, POAG was defined as age of diagnosis >35, intraocular pressure greater than 22mm Hg in both eyes, glaucomatous optic nerve damage, and corresponding visual field loss in a least one eye. Phenotypic stratification gave increased evidence for linkage in individuals with an age of diagnosis \geq 45 in four adjacent markers (GABRB3, d15s822, d15s217, d15s165) spanning chromosome 15q11.2-15q13.2, (Allingham, et al 2001). Candidate genes were selected throughout this region based on position, function and availability of completed sequence data from the UCSC genomic assembly. The coding regions of these genes (ATP10C, GABRA5, GABRB3, GABRG3, APBA2, Kiaa0574, TJP1) were sequenced to identify mutations and polymorphisms. A sample set of 14 individuals from 11 families and 2 control samples, were included in the screen. Intron/exon boundaries were determined by BLAT comparison of the cDNA sequence and the UCSC genomic assembly (<http://genome.cse.ucsc.edu>). Primers were designed flanking each exon, PCR amplification was performed, and the sequence reactions were run on an ABI3700, following standard protocols. Sequences were assembled into contigs and analyzed using Sequencher (Gene Codes Co., Ann Arbor, MI). Several SNPs, silent mutations, and missense mutations were identified throughout the genes. None of the missense mutations identified clearly segregated with disease in the families screened, indicating that none of these genes play a primary causative role in POAG. Further association studies, utilizing the SNPs and mutations we have identified, are underway.

Are Single-Nucleotide Polymorphisms of N-acetyltransferase Genes (NAT1 and NAT2) associated with Parkinson Disease? *F. Zhang^{1,2}, J. van der Walt^{1,2}, E. Martin^{1,2}, B. Scott^{1,2}, J. Haines², M. Nance², J. Hubble², W. Koller², R. Pahwa², M. Stern², B. Hiner², J. Jankovic², C. Goetz², F. Small², R. Gibson², L. Middleton², M. Perikak-Vance^{1,2}, J. Vance^{1,2}.* 1) Duke University, Durham, NC; 2) Duke CHG/GlaxoSmithKline PD Genetics Collaboration.

Parkinson Disease (PD) is a common neurodegenerative disease. There is evidence that the development of PD may be affected by multiple environmental and genetic factors. Reports on the association of N-acetyltransferase 2 with PD are inconsistent. Recently, our results from a complete genomic screen have shown a linkage of late-onset PD to a region of chromosome 8p, where the N-acetyltransferase genes NAT1 and NAT2 are located. To provide further evidence for the role of NAT1 and NAT2 in PD, we conducted a family-based association study on these two genes. We studied 1580 individuals from 397 families with at least one affected. Families were stratified as family history positive versus negative, and late age of onset (>40 years) versus early age of onset. Single nucleotide polymorphisms (SNPs) were genotyped for NAT1 (positions 445, 560, 640, 1088, and 1095) and NAT2 (positions 282, 341, 481, 590, 857). Hardy-Weinberg equilibrium (HWE) and pairwise linkage disequilibrium (LD) tests were examined for SNPs in each gene. Data were analyzed using the pedigree disequilibrium test (PDT) and the likelihood ratio test of Transmit. SNP haplotypes within and across NAT1 and NAT2 were tested for association using haplotypes with frequency > 2%. No evidence for deviation from HWE was found. In each gene the pattern of LD between affected and unaffected people is similar. In overall and stratified data sets, both PDT and Transmit failed to show any statistical significance for single loci. Neither did five-SNP haplotypes for either gene or six-SNP haplotypes across NAT1 and NAT2. This represents one of the most comprehensive investigations of these genes in PD in a very large family data set. Based on the results, we conclude that NAT1 and NAT2 are not significant genetic risk factors in PD.

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Functional Analysis of the Regulation of Dopamine Transporter Gene Expression. *T.A. Greenwood, J.R. Kelsoe.*
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A role for the human dopamine transporter (DAT) gene in bipolar disorder is implicated by several lines of pharmacological evidence, as well as suggestive evidence of linkage at this locus. TDT analysis of a sample of 50 parent-proband triads from our UCSD/UBC/UC family collection revealed a particular haplotyped marker deriving from the 3' region of the DAT gene (exon 9 through exon 15) to be in strong linkage disequilibrium (LD) with bipolar disorder ($p=0.0004$). Subsequent analysis of an additional 70 triads from the NIMH Genetics Initiative for Bipolar Disorder collection revealed more modest evidence for association to a region slightly 5' of that originally implicated (intron 2 through intron 10). As these two regions exhibit an overlap defined by four polymorphisms deriving from exon 9, intron 9, and intron 10, it is possible that a functional variant contributing a susceptibility to bipolar disorder exists within or near this region. However, LD analyses did not enable us to more precisely localize the putative functional variant. In order to more definitively identify the region containing the putative regulatory element, we have analyzed the effect on expression of a number of the potential regulatory and intronic regions within the DAT gene. These analyses indicate a strong core promoter and potential repressor elements in both the proximal 5' flanking region and intron 1. A 1.5-fold difference in regulatory activity was also observed between the different haplotypes of these promoter segments, representing the two 5' clades. We found no effect on transcription with inclusion of the 9- and 10-repeat alleles of the 3' VNTR. However, introns 9, 12, and 14 appear to contain enhancer elements capable of increasing expression approximately 2-fold with respect to the promoter constructs. There also appear to be differences in expression between the two alleles of intron 14, as well as between haplotypes comprised of different alleles of the promoter and exon 9/intron 9. These results thus indicate that it may be the particular combination of polymorphisms in a haplotype across the gene that ultimately effect DAT gene expression.

Pseudo-dominant mode of inheritance in Thomsen's myotonia congenita. *N.H. Mao, F. Lehmann-Horn, K. Jurkat-Rott.* Applied physiology, University of Ulm, Ulm, Germany.

Myotonia is skeletal muscle stiffness due to delayed muscle relaxation. Two entities of myotonia have been described : Thomsen's myotonia with dominant inheritance (dominant myotonia congenita, DMC) and Becker's myotonia with recessive inheritance (recessive generalized myotonia, RGM). Mutations in the gene *CLCN1* encoding the skeletal muscle chloride channel are responsible for both DMC and RGM. To date, more than 60 mutations have been identified. Because of the clinical similarities and lack of information on other family members among DMC, RGM and other diseases, it is useful to develop a simple method for mass screening of mutations in *CLCN1* to facilitate genetic diagnosis. By single strand conformation analysis (SSCA), we identified 11 novel mutations. Then by multiplex allele-specific PCR (ASPCR), we screened for these 11 novel mutations and other 43 known mutations in 82 unrelated families with DMC and 167 unrelated families with RGM. In RGM families, 24% of all families were clarified on both alleles and additional 37% on one allele. In the German population, the most frequent mutations were R894X, fs503X and F413C with prevalences of 35%, 16% and 8.9% respectively. Comparatively, F167L, R894X and T268M were most three frequent mutations in the Turkish population with prevalences of 13%, 9% and 4.5% respectively. One frequent mutation A531V found in Finnish population wasn't present in our sample set. In DMC families, 14.6% of all families had dominant mutations and 15.8% had recessive mutations in *CLCN1*. The reason for this may be because some recessive mutations in *CLCN1* are so frequent that heterozygous spouses occur in several subsequent generations resulting in a pseudo-dominant inheritance. With the technique ASPCR, fifty four mutations can be tested for in only 14 PCR reactions for 192 samples in 14 working days, which is at least required 32 working days by SSCA. From the point of view of time and money, ASPCR is a powerful method for mass screening.

Sequence variation in 3 human homologs of the *C. elegans daf-16* gene: candidate genes for human longevity.

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It has been estimated that 25% of the variance in human longevity is attributable to genetic components. In model organisms such as the worm *C. elegans*, the insulin/IGF1 signaling pathway has been shown to be important in determining life span. In worms, the insulin receptor is encoded by the *daf-2* gene and mutations in this gene have been associated with increased longevity in worms. This increase in longevity has been shown to require the forkhead transcriptional protein, encoded by the *daf-16* gene. In humans, there are three homologs of the *daf-16* gene: FKHRL1, FOXO1A, and AFX1. There is currently no comprehensive polymorphism information for any of these three genes. In order to determine whether mutations in these genes might underlie some of the variation in longevity in humans, we are scanning these three genes for polymorphisms in a sample of 100 individuals. A minimal set of polymorphic sites representing the maximum amount of information will be chosen to use for case-control studies where cases are individuals who are exceptionally long-lived, and controls are individuals who died at or earlier than the median age of death of non-traumatic causes.

Mice lacking podocin develop a severe glomerular phenotype. *S. Roselli*¹, *M. Sich*¹, *M.C. Gubler*¹, *C. Antignac*^{1,2}. 1) Inserm U423, Necker Hospital, Paris, France; 2) Department of Genetics, Paris 5 University, Necker Hospital, Paris, France.

NPHS2, encoding the slit diaphragm associated protein podocin, is implicated in one form of autosomal recessive steroid-resistant nephrotic syndrome characterized by early childhood onset of proteinuria, rapid progression to end stage renal disease and focal segmental glomerulosclerosis (FSGS). In order to study the exact podocin function and to better understand the pathophysiology of nephrotic syndrome, we generated a mouse model by inactivating *Nphs2* in embryonic stem cells by homologous recombination. A 4 kb genomic region spanning exons 1 and 2 and including the predicted transcription start site was replaced by a PGKHyg cassette containing the hygromycin resistance gene. Here we show preliminary results based on the analysis of 2 litters born from heterozygote matings. Three out of 12 newborn mice were homozygous for the inactivated *Nphs2* allele indicating that the *Nphs2* ^{-/-} genotype does not lead to massive embryonic lethality. Although seemingly normal at birth, the 3 *Nphs2* ^{-/-} mice rapidly present with growth retardation in the first days of life and died at 4, 7 and 10 days respectively. Massive proteinuria was found in the urine collected in the bladder of the 7 day-old mouse. In this mouse, glomerular lesions were severe and diffuse, involving not only the mature glomeruli but also the immature ones in the subcapsular region. They consist in diffuse enlargement of the mesangial matrix without cell proliferation resulting focally in segmental or global sclerosis of the tuft. No capsular adhesion was seen. Altogether, these data show that *Nphs2* ^{-/-} mice develop a severe glomerular disease with early occurrence of diffuse mesangial sclerosis. This phenotype is far more severe than the one observed in humans with *NPHS2* mutations. These results confirm the essential role of podocin in the maintenance of a functional kidney filter.

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The murine *klotho* gene coding sequence is completely conserved in laboratory-derived inbred strains. A. Bektas, C.A. Francomano. Laboratory of Genetics, National Inst on Aging/NIH, Baltimore, MD.

The *klotho* gene is involved in the suppression of several aging phenotypes, based on the *klotho* mutant mouse. A defect in *klotho* gene expression in the mouse results in a syndrome that resembles human aging, with premature death, osteoporosis, atherosclerosis, diabetes, and emphysema. In an effort to find functional murine variants of *klotho*, we sequenced the gene from genomic DNA of 20 different mouse strains from the Jackson Laboratory phenome project. The panel includes 16 laboratory-derived inbred strains, and 4 wild-derived inbred strains. Among the 16 laboratory derived strains, no sequence variation was found in any of the exons or intron-exon boundaries. Among the 4 wild-derived strains on the panel, we found 43 sequence variants in the coding region as well as in the introns. Only 5 variations resulted in amino acid substitutions. These included: P11Q, S117L, A446G (SPRET/Ei); A567V, H615Y (CAST/Ei); and H615Y (MOLF/Ei). We also found a deletion in intron 1 in SPRET/Ei and an insertion in intron 4 in CAST/Ei and MOLF/Ei. These observations suggest a very high degree of selection against sequence variation in the *klotho* gene. The functional consequences of sequence variation in the wild-derived strains are under investigation.

Identification of novel variations within IL-4 and association with asthma and associated phenotypes in a Dutch population. *M.J.M. Basehore¹, E.R. Bleecker¹, T.D. Howard¹, H. Jongepier², D.A. Meyers¹, D.S. Postma².* 1) Center for Human Genomics, Wake Forest Univ Sch of Med, Winston-Salem, NC; 2) Dept of Pulm, Univ Hospital, Groningen, the Netherlands.

Asthma is a common respiratory disease characterized by acute and chronic bronchial inflammation and intermittent airway obstruction. Findings closely associated with the asthma phenotype include bronchial hyperresponsiveness (BHR) and total serum IgE levels, both of which have a strong genetic component. Asthma and/or atopy susceptibility genes have been mapped to a region on chromosome 5q31-q33 in multiple populations. There is strong evidence that one or more loci within this region is closely associated with BHR and elevated serum IgE levels, the two most characteristic clinical phenotypes of asthma. A cluster of proinflammatory cytokine genes important in immune regulation resides within this candidate locus, including interleukin (IL)4 and IL-13. In the present study, we identified 21 new sequence variants in the IL-4 gene by re-sequencing 8 Dutch probands with asthma and 8 Dutch controls. We performed association studies with 12 of these polymorphisms in a Dutch population (originally ascertained by a proband with asthma) that has previously shown evidence for linkage to chromosome 5q31 for BHR and total serum IgE levels. We have also evaluated the interaction of IL-4 polymorphisms with other previously identified polymorphisms in both IL-13 and IL-4 receptor alpha. Five of the identified novel IL-4 polymorphisms showed significant association with asthma or an asthma-related phenotype. Significant associations were observed with % predicted FEV1, change in post-bronchodilator FEV1 over an extended time period and skin test positivity. These data suggest that variations within the IL-4 gene may contribute to both asthma susceptibility as well as disease severity.

Sequence analysis of promoter region of the brain-derived neurotrophic factor gene in parkinson's disease. A. Parsian¹, R. Sinha¹, B. Racette², J. Perlmutter². 1) Birth Defects Ctr, Univ Louisville Hlth Sci Ctr, Louisville, KY; 2) Dept of Neurology, Washington Univ Med Sch, St. Louis, MO.

Idiopathic PD is one of the most common neurodegenerative disorders affecting about 1% of people over the age of 60 year. Most cases of PD are sporadic but approximately 20% have a positive family history suggesting a hereditary component. Although most of the genetic factors are unknown, genes coding for nerve growth factors involved in dopamine receptor and cellular regulation such as Brain-derived neurotrophic factor (BDNF) are logical candidate genes. To determine the role of the BDNF gene in the development of familial and sporadic Parkinson's disease (PD), we sequenced the promoter region of the gene using genomic DNA from 45 patients with familial PD to identify unique mutation or variation. Two single nucleotide polymorphisms (SNPs) at positions C-1331T and C270T were identified. There was no sign of linkage disequilibrium between these two SNPs. We screened our entire sample of PD with the SNP at C270T in the 5' non-coding region of the gene. The sample comprised of 146 patients with and 208 without a positive family history for PD and 195 matched controls. The allele frequencies in the familial PD subjects were significantly different compared to normal controls ($p < 0.0006$) but not significantly different between sporadic PD and normal controls. Both PD groups (familial and sporadic) were categorized based on age of onset (AON) of 45 years into two subgroups of young (<45) and typical (>45). The allele frequencies of the familial group with AON of >45 were significantly different than normal controls but not the sporadic groups or familial with AON of <45. In addition, there were only three patients who were homozygous for the T allele. Our data indicate the possibility of linkage disequilibrium between this variation and a mutation in coding region of BDNF gene. Our findings suggest that BDNF may play a role in familial PD with typical AON and warrant further study.

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Differential screening of gene initiation sequences using polymerase chain reaction. *X. Ni, J. Trakalo, G. Wong, J. Kennedy.* Neurogenetics Section, Ctr Addiction & Mental Health, Toronto, ON, Canada.

Purpose: Gene initiation sequences (GIS) contribute significantly to gene expression both in vivo and in vitro. Effective methods are needed to identify variation in the GIS that can lead to differential expression of coding sequences. This report describes a method to screen for GIS differences between patients with major psychoses versus controls using the polymerase chain reaction (PCR). **Methods:** The key element is to use the consensus Kozak sequences with the addition of one or two 3 bases as the forward anchoring primers for gene initiation sites. We designed 40 anchoring primers; 32 primers for downstream sequences and 8 for upstream sequences. Reverse primers were chosen at random from a set of arbitrary primers. Individual samples and pooled DNAs were used to screen for variants. After amplification with an optimal protocol, products were separated using polyacrylamide gel electrophoresis. Differential DNA bands were excised from the gel and sequenced. **Results:** We have identified 201 DNA bands which show either different frequencies between patients with schizophrenia or bipolar disorder and controls in individual samples, or differing banding patterns in pooled DNAs. Fifty-four DNAs have been sequenced and 5 candidate genes, 36 clones, and 13 unknown sequences from the human genome have been identified. **Conclusions:** Our results demonstrate the potential of this technique to identify variations in the GIS. It should be possible to detect most of the GIS variants in an individual by use of multiple primer sets. Thus our GIS methods should be quite valuable for testing gene expression hypotheses in schizophrenia and bipolar disorder. **Key words:** gene initiation sequence, gene expression, differential screening, polymerase chain reaction *This work is supported by CIHR/CPRF partnered Fellowships.

Bardet-Biedl Syndrome and its relationship to QTL for obesity in the Newfoundland population. *Y. Fan¹, E. Dicks², J.S. Green², P.S. Parfrey², W.S. Davidson¹.* 1) Dept Molecular Biol, Biochem, Simon Fraser Univ, Burnaby, BC, Canada; 2) Faculty of Medicine, Health Sciences Centre, Memorial University, St. John's, NF, Canada.

Bardet-Biedl Syndrome (BBS) is characterized by retinal dystrophy, polydactyly, hypogonadism, learning difficulties, renal dysplasia, and obesity. Although it has traditionally been considered that BBS is inherited in an autosomal recessive fashion, there is evidence for triallelism and it may serve as a bridge between Mendelian and multifactorial inheritance. There is a high incidence of BBS in the Newfoundland population that cannot be explained by a single ancestral founder event. We considered the possibility that the increased incidence of BBS is due to an underlying common modifier gene in this population. It has been suggested that there is a link between BBS and obesity in the general public. Therefore, we examined the relationship between QTL that have been shown to influence obesity and BBS loci in 13 families. Genotypes were constructed for the obesity-related QTL on chromosome 13q14 and chromosome 7q32.3. Comparisons of the haplotypes of the obesity-related QTL revealed a few groups of partially related chromosomes among the different families. However, there was no obvious relationship between any of the obesity-related QTL haplotypes and the five BBS loci represented in the 13 families. This suggests that the molecular basis of obesity in BBS individuals in the Newfoundland population is unrelated to the mechanism resulting from the obesity-related QTL on chromosomes 7 and 13. (Supported by the Canadian Institutes of Health Research).

Dinucleotide Repeat Expansion and Clonal Selection of Mesangial Cells in Diabetic Nephropathy. *A. Fornoni, O. Lenz, A. Banerjee, G.E. Striker, L.J. Striker.* Vascular Biology Institute, Department of Medicine, University of Miami School of Medicine, Miami, FL.

Clonal expansion of cells with a disease phenotype characterizes chronic diseases such as atherosclerosis and systemic sclerosis. We propose that a similar pathogenetic mechanism occurs for progressive diabetic glomerulosclerosis. We used the ROP mouse strain prone to progressive glomerulosclerosis to investigate the influence of 25 mM glucose in vitro and diabetes in vivo on dinucleotide repeat instability and clonal selection of mesangial cells. We used the length of the dinucleotide repeat of the matrix metalloproteinase-9 (MMP-9) promoter as a marker to discriminate different mesangial cell clones. We found that mesangial cells (MC) isolated from ROP mice consisted of clones that are heterogeneous in the length of the d(CA) repeat in the MMP-9 promoter. Using PCR and automated sequencing we detected sub-populations with either 20 or 24 d(CA) repeats. No such heterogeneity could be detected in MC isolated from sclerosis-resistant C57BL/6 mice. 25 mM glucose and diabetes selected for clones with 24 d(CA) repeats only. Analysis of single cell clones showed that two mechanisms were responsible for induction of clones with 24 d(CA) repeats: d(CA) repeat expansion and clonal selection. The mesangial cell changes induced by 25 mM glucose in vitro were reversible, whereas those induced by diabetes in vivo were not. We conclude that the presence of heterogeneous clones of MC may be a marker for the susceptibility to develop progressive glomerulosclerosis. Genomic changes induced by elevated glucose concentrations suggest a novel mechanism for the pathogenesis of diabetic nephropathy.

Comprehensive Detection of CFTR Mutations in Idiopathic Chronic Pancreatitis. *J. Feng¹, J.A. Cohn^{2,3}, J.P. Neoptolemus⁴, Z. Jiang¹, W. Greenhalf⁴, I. Ellis⁵, S.S. Sommer¹.* 1) Dept of Molecular Genetics, City of Hope National Med Ctr, Duarte, CA; 2) Dept of Medicine, Duke Univ, Durham, NC; 3) V.A. Med Ctr, Durham, NC; 4) Dept of Surgery, Univ of Liverpool, Liverpool, UK; 5) Alder Hey Children's Hosp, Liverpool, UK.

BACKGROUND: Idiopathic chronic pancreatitis (ICP) accounts for most chronic pancreatitis in children and nonalcoholic adults. Some ICP patients are CFTR compound heterozygotes who have a CF-causing mutation plus a milder mutation (*Gastroenterology* 121:1310-1319, 2001). This study examined a large series of ICP patients for rare CFTR mutations to determine whether ICP risk is increased by CF carrier genotypes containing one normal CFTR allele. **METHODS:** Fifty-five sporadic cases of ICP were recruited by the European Registry of Hereditary Pancreatic Diseases (EUROPAC). Most cases (75%) were British and patients with cationic trypsinogen mutations (causing hereditary pancreatitis) were excluded. DNA was tested for CFTR mutations by DOVAM-S, a highly multiplexed and robotically enhanced form of SSCP that detects virtually all mutations in the tested regions, as validated by blinded analyses of samples containing 250+ different mutations in other genes. DNA sequencing was used to confirm the presence of one normal CFTR allele in selected CF carriers. **RESULTS:** At least one abnormal CFTR allele was detected in 21/55 tested ICP patients. In this series, ICP risk was strongly associated with CFTR compound heterozygosity and it was also associated with CF carrier genotypes and with the R75Q mutation. **SUMMARY:** Comprehensive testing of a large series of ICP patients confirmed the strong association of ICP with CFTR compound heterozygosity and it also associated ICP with CF carriers who have one normal CFTR allele. The latter finding is unexpected because it implies that 50% loss of CFTR function is sufficient to increase the risk of ICP. (Supported in part by a V.A. Merit Review Grant).

Polymorphism of interleukin ligands and receptors genes and variability of quantitative asthma-associated traits.

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In 323 Russian patients with atopic bronchial asthma and their relatives, inhabitants of Tomsk, Russia, a contribution of eight single nucleotide polymorphisms of interleukin ligands and receptors genes (IL4, IL5, IL9, IL4RA, IL5RA, IL5RB) in a variation of quantitative asthma-associated traits (lung volumes, bronchial reactivity, common serum immunoglobulins levels) was investigated. It was established, that polymorphisms of the genes of study have a significant contribution to variability of the given complex of the traits and this effect differs by force and direction for different genes and attributes. The mean percent of general phenotypic variance of the quantitative traits explained by a genetic variety on the set of genetic loci was 1.63-5.54% in men and 1.03-2.15% in women. The percent of the contribution of variability on analyzed loci to general polygenic variance of the traits was 3.96-32.11% and 2.60-12.23% in men and women, respectively. The obtained data testify, that interleukin ligands and receptors genes polymorphisms are only a part of a hereditary diversity determining a variability of quantitative risk factors for atopic bronchial asthma.

Multiple sclerosis (MS) in DR2 homozygotes: dose effect of HLA genes on susceptibility and influence on disease course. *L.F. Barcellos¹, J.R. Oksenberg¹, R.R. Lincoln¹, S. Schmidt², P. Bucher¹, A. Swerdlin¹, A.B. Begovich³, E. Vittinghoff¹, M.A. Pericak-Vance², J.L. Haines⁴, S.L. Hauser¹ and Multiple Sclerosis Genetics Group.* 1) University of California, San Francisco, CA; 2) Duke University Medical Center, Durham, NC; 3) Roche Molecular Systems, Alameda, CA; 4) Vanderbilt University, Nashville, TN.

The etiology of MS is complex, involving both genetic and environmental components. Linkage to the HLA-DR locus, and association with the DR2 haplotype (DRB1*1501-DQB1*0602) within the MHC on ch.6p21 have been consistently demonstrated. Approximately 70% of familial and 50% of sporadic MS cases have at least one DR2 allele compared to 28% of control subjects. This finding implicates DR2 as an important genetic factor in the majority of MS cases. We examined the effect of DR2 gene dose in 549 stringently ascertained Caucasian MS families (187 multicase and 362 singleton families; total n=2382 comprised of 808 patients and 1574 unaffected family members) and show that it is correlated with increased risk and disease course. All individuals were stratified by HLA-DR2 genotype (DR2/DR2, DR2/DRX and DRX/DRX, where X denotes other DR alleles). 52.8% (n=94) DR2/DR2 individuals were affected, in contrast to 38.7% (n=395) DR2/DRX and 26.9% (n=319) DRX/DRX individuals. Using DRX/DRX individuals as the reference group, significant results were obtained from conditional logistic regression modeling when patients were compared to unaffected family members for both DR2/DR2 and DR2/DRX genotypes (OR=6.7, $p < 10^{-6}$ and OR=2.7, $p < 10^{-6}$, respectively). Significant evidence for a dose effect was present and suggests that two copies of DR2 confer an even greater disease risk relative to one copy (OR=2.5, $p < 10^{-5}$, OR=2.4, $p < 10^{-3}$, OR=2.7, $p < 0.01$ for all families, multicase, and singleton families, respectively). DR2 homozygotes were significantly less frequent in patients with a more benign disease course (EDSS <3 after 10 or more years; 5.4% vs. 17.3%, OR = 0.3, $p = 0.037$), providing evidence to support a disease modifying role for HLA-DR2. These important findings shed new light on our understanding of the complex molecular mechanisms that underlie MS pathogenesis.

Role of serotonin transporter variants in the determination of platelet serotonin levels in Autism Spectrum

Disorders. *A.M. Coutinho¹, S. Silva¹, C. Fesel¹, T. Morgadinho³, T.R. Macedo³, C. Bento², C. Marques², A. Ataíde², T. Miguel², G. Oliveira², A.M. Vicente¹.* 1) Instituto Gulbenkian Ciência, Oeiras, Oeiras, Portugal; 2) Hospital Pediátrico de Coimbra, Coimbra, Portugal; 3) Faculdade de Medicina da Universidade de Coimbra, Coimbra, Portugal.

The role of the serotonin system in the etiology and pathogenesis of autism spectrum disorders (ASD) is not clearly defined. High levels of platelet serotonin (5-HT) are found in a proportion of patients, and the serotonin transporter (5-HTT) has been associated to autism in some studies. The relation between these two findings and with autism etiology is still not clear. We have examined the association of platelet serotonin levels with two 5-HTT polymorphisms (5-HTTLPR in the promoter and Stin2 in intron2) in a sample of 106 ASD patients, their parents, and 52 age-matched controls. The heritability of 5-HT levels was estimated at 83%, although a positive father/mother correlation shows that a nuclear environment effect is present. The association of this trait with 5-HTT was tested using the quantitative transmission disequilibrium test (QTDT), and a significant association was found with the long/Stin2.10 haplotype ($p=0.0012$). The association between this 5-HTT haplotype and 5-HT levels was confirmed using ANOVA, and emphasized in long/Stin2.10 homozygous individuals ($p=0.0049$). 70% of the alleles in hyperserotonemic patients were long/Stin2.10, against 25% in normal 5-HT level patients ($p=0.006$, OR 7.1). This study clearly shows a role of 5-HTT alleles in the determination of 5-HT levels. A direct relation of 5-HT levels with ASD could not be established since its distribution in patients was not significantly different from controls, although a trend towards higher levels was observed in the patients sample. We found no transmission disequilibrium of any of the 5-HTT variants with ASD. However, the excess of the long/Stin2.10 haplotype in hyperserotonemic patients suggests that these may represent a separate etiological group. Given the heterogeneity of ASD, further exploration of this question is warranted.

Crohn's Disease: Role of CARD15/NOD2 Gene Mutations in Clinical Heterogeneity. *S.R. Brant¹, M.F. Picco², J.P. Achkar³, S. Kane⁴, T.M. Bayless¹, F. Nouvet¹, D. Bonen⁴, A. Brzezinski³, A. Karban¹, T. Dassopoulos¹, R. Karaliukas⁴, D. Vogel³, T.H. Beaty⁵, S.B. Hanauer⁴, R.H. Duerr⁶, J.H. Cho⁴.* 1) Dept Med, Johns Hopkins Univ, Baltimore, MD; 2) Dept Med, Mayo Clinic, Jacksonville, FL; 3) Dept Gastro, Cleveland Clinic Foundation, Cleveland, OH; 4) Dept Med, Univ of Chicago, Chicago, IL; 5) Dept Gen Epidemiology, JHU Sch Pub Hlth, Baltimore, MD; 6) Dept Med, Human Genetics, Univ of Pittsburgh, Pittsburgh, PA.

Crohn's disease (CD) is a chronic intestinal inflammatory disorder, highly variable in phenotypes of age at diagnosis, intestinal site, fistulizing or stricturing behavior, and need for surgery. CARD15 gene mutations L1007fsinsC, G908R, and R702W are established molecular genetic CD risk factors. We examined effect of CARD15 mutations on phenotype in context of other established CD risk factors. **METHODS:** Age at diagnosis, family history, Jewish ethnicity, tobacco use, surgery, disease site and behavior were determined by questionnaires, interviews and case-record reviews in 275 unrelated patients with CD. The 3 CARD15 mutations were genotyped by ARMS assay. Correlations were analyzed by univariate (chi-sq. or Fishers) and multivariate (linear regression and Cox proportional hazards) methods. **RESULTS:** There were 32 homozygotes or compound heterozygotes (MM), 76 heterozygotes (MW), and 167 wildtype patients (WW). MM patients had a younger age at diagnosis than MW or WW patients (mean 21.4 vs. 24.4; $p=0.03$), more ileal disease involvement (MM 97%, MW/WW 77%; $p=0.01$), and more complications of strictures (Odds Ratio, OR 4.1; $p=0.03$) and non-perianal fistulas (OR 5.0; $p=0.004$) (relative to patients with neither complication). By logistic regression MM genotype was the strongest significant risk factor for ileal disease (OR 10.1) followed by smoking at diagnosis (OR 2.25) and age at diagnosis (OR 0.97 per increased year). Ileal sites (OR 4.8) and carrying CARD15 mutations (OR 1.9 MW and 3.5 MM) were independent risk factors for stricturing or fistulizing behavior. Ileal disease, youthful onset and smoking at diagnosis (but not CARD15 mutations) were risk factors for early surgery. Therefore, CARD15 mutations are important determinants of CD phenotypic heterogeneity.

MUTATIONS IN THE COL4A4 GENE IN RELATION TO FAMILIAL HEMATURIA. *D. Glavac¹, M. Slajpah¹, A. Meglic², M. Ravnik-Glavac^{1,3}.* 1) Department of Molecular Genetics, Medical Faculty, Institute of Pathology, Korytkova 2, 1000, Ljubljana, Slovenia; 2) Department of Paediatrics, University Medical Centre, Ljubljana, Slovenia; 3) Institute of Biochemistry, Medical Faculty, Ljubljana, Slovenia.

Mutations in COL4A4 gene located at 2q35, have been reported in autosomal recessive and dominant type of Alport syndrome (AS), as well as benign familial hematuria (BHF) or thin basement membrane disease (TBMD). BHF probably affects at least 1% of the population and is characterized by persistent microscopic glomerular hematuria, sometimes with proteinuria or hypertension, thinning of the glomerular basement membrane (GBM) and normal renal function. Considering the similarities in GBM abnormalities, TBMD cannot be clinically differentiated from the initial stages of AS. It has recently been suggested that TBMD represents a carrier state of autosomal recessive or X-linked AS. We analyzed 22 patients with confirmed BHF and 12 AS patients who had tested negative in COL4A5 gene screening also for COL4A4 mutations. Non-isotopic single stranded conformation analysis (SSCA) after amplification of each exon with boundary intronic sequences by the polymerase chain reaction (PCR) was used for all 51 exons of COL4A5 and 47 exons of COL4A4 gene. While no mutation was identified in COL4A5 gene in all 34 patients COL4A4 mutation screening disclosed eleven common polymorphisms that are shared in part with other populations and six novel mutations: G774R (GGT>CGT), G789G (GGA>GGT), 2860+2T>G, R908W (CGG>GGG), D1049H (GAC>CAC)+del 5 bp, 3506-8T>G and 4081-8G>C comprising three missense mutations, one frameshift and two potentially splice site mutations. Furthermore, we found one silent mutation, one rare variant in a non-coding region, and several polymorphism with a very low heterozygosity. This study confirms the importance of the COL4A4 gene in the pathogenesis of benign familial hematuria.

A novel C597A polymorphism of Norrie disease gene is associated with Retinopathy of prematurity in Kuwaiti Arabs. *M.Z. Haider¹, L.V. Devarajan¹, M. AlEssa¹, H. Kumar²*. 1) Dept Pediatrics, Fac Medicine, Kuwait Univ, Safat, Kuwait; 2) Dept Ophthalmology, Ibn Sina Hosp, Sulaibikhat, Kuwait.

Retinopathy of prematurity (ROP) is a retinal vascular disease which occurs in infants with a short gestational age and low birth weight and may lead to retinal detachment and blindness. In some premature infants, ROP progresses to advanced stages despite rigorous intervention, but in the majority, it spontaneously regresses before the threshold stage. Genetic factors, e.g., mutations in the Norrie disease (ND) gene have been implicated in determining the progression of ROP to advanced stages. We have identified a novel C597A polymorphism and screened this in 210 Kuwaiti premature infants using PCR-RFLP and DNA sequence analysis, to investigate an association with ROP. In our Kuwaiti premature newborn cohort, 115 of 210 babies had no eye problems and served as controls while 95 were found to have ROP. In 71 of the 95 ROP cases, the disease spontaneously regressed at or before stage 3, while in 24 of 95 ROP cases, the disease progressed to advanced stages 4 or 5. The incidence of the AA genotype of the C597A polymorphism was considerably higher in advanced stage ROP cases (83.3%) compared to 0 in spontaneously regressing ROP cases and 10.4% in the normal controls ($p < 0.0001$). For other genotypes, no significant difference was detected between the controls and ROP cases. The data show a strong association of this novel C597A polymorphism of the ND gene, with progression of ROP to advanced stages.

Maternal DBH levels modulate obsessive-compulsive behaviors in autistic probands. *R. Mankoski^{1,2}, M. Dowd¹, K. Smith², S. Folstein^{1,2}.* 1) Psychiatry, New England Medical Center, Boston, MA; 2) Genetics Program, Sackler School of Graduate Biomedical Sciences, Tufts University, Boston, MA.

Autism is a neurodevelopmental disorder characterized by abnormal social interactions, abnormal language, and restricted and repetitive behaviors. Dopamine beta-hydroxylase (DBH) converts dopamine (DA) to norepinephrine (NE) and is postulated to play an epigenetic role in the etiology of autism by modulating prenatal intrauterine concentrations of DA and NE. DBH levels are thought to be controlled in part by two promoter polymorphisms: a 19 base-pair insertion/deletion (D19) and a c/t transition (-1021 c/t). The D19 +/+ and -1021 c/c alleles are associated with high DBH activity. 100 mothers and 94 fathers of autistic subjects were genotyped for each polymorphism. Genotype distributions were evaluated with a chi-square test using the fathers as controls. 6 Autism Diagnostic Interview-based factors (each scored 0-1) were compared between children of D19 +/+ vs. D19 +/- and -1021 c/c vs. -1021 t/t mothers using two-tailed t-tests. The genotype distribution of the D19 alleles (+/+, +/-, -/-) is different in mothers compared to fathers ($p = 0.02$). This appears to be due to an increased prevalence of the +/+ allele (+/+ vs. +/- and -/-, $p = 0.005$). There is no difference in the distribution of the -1021 c/t alleles (c/c, c/t, t/t) in mothers compared to fathers ($p = 0.09$). Autistic children of D19 +/+ mothers score 1.6 times worse on measures of obsessive-compulsive behaviors than children of D19 +/- mothers (0.33 vs. 0.21, $p = 0.001$). Children of -1021 c/c mothers score 1.6 times worse on measures of abnormal spoken language than children of -1021 t/t mothers (0.45 vs. 0.28, $p = 0.02$), although this is not significant after correction for multiple tests. We conclude that DBH promoter alleles associated with high maternal DBH activity lead to worse outcomes with respect to obsessive-compulsive behaviors and possibly spoken language. The D19 +/+ allele appears to have a higher prevalence than expected in mothers of autistic probands. Low intrauterine DA may be a risk factor or low NE may be a protective factor for aspects of the autism phenotype.

Possible association of serotonin 1B receptor and cholecystokinin 1 receptor gene polymorphisms in bipolar and unipolar affective disorder cases in Estonia. A. Kurg¹, T. Nikopensius¹, P. Hallast¹, M. Laan¹, S. Kõks², J. Slik³, E. Maron³, E. Vasar², A. Metspalu¹. 1) Dept. of Biotechnology, Inst. of Molecular & Cell Biol, Univ. of Tartu / Estonian Biocentre Tartu, Estonia; 2) Dept. of Physiology, University of Tartu, Tartu, Estonia; 3) Clinic of Psychiatry, University of Tartu, Tartu, Estonia.

Human mood disorders have complex inheritance, involving the interaction of multiple genes in combination with environmental factors. So far linkage and association studies searching candidate genes for mood disorders have yielded controversial results, leading to multiple nonreplications in second-generation genomic screens. Many studies have focused on relationship of polymorphisms in genes related to two major - serotonergic and dopaminergic - neurotransmission systems, expected to play an important role in the genetic predisposition to neuropsychiatric disorders. We have performed a scan of single nucleotide polymorphisms (SNPs) selected from a total of 24 candidate genes including majority of serotonin-, dopamine- and cholecystokinin-related genes, endogenous opioid system genes and wolframin gene. In a subsequent pilot association study we genotyped 212 polymorphisms with Arrayed Primer EXTension (APEX) technology in a sample of 73 unrelated patients with unipolar or bipolar (DSM-IV) disorder and 72 matched controls from Estonian population. Case-control comparison of allelic and genotypic distribution demonstrated significantly higher frequencies of mutant alleles and enriched fraction of minor allele homozygotes in patient group with polymorphisms 129C-T in serotonin 1B receptor gene (29% vs 18%, $P=0.05$) and 1266T-C in cholecystokinin 1 receptor gene (20% vs 9%, $P=0.01$). Further studies with larger sample size should provide evidence, if these associations are true positives. We will investigate whether these markers present real functional polymorphisms, or they are in LD with a true functional polymorphisms in the same gene or nearby allelic variant playing role in determining susceptibility to depressive illnesses.

NOD2 genotype-phenotype correlations in Canadian Crohns Disease families. *W.G. Newman^{1,2,4}, A.H. Steinhart^{1,4}, R. McLeod^{3,4}, G. Greenberg^{1,4}, Z. Cohen^{3,4}, M. Silverberg^{1,2,4}, K.A. Siminovitch^{1,2,4}.* 1) Dept of Medicine, University of Toronto, Canada; 2) Dept of Immunology, Medical Genetics and Microbiology, University of Toronto, Canada; 3) Dept of Surgery, University of Toronto, Canada; 4) Mount Sinai Hospital, Toronto, ON, Canada.

Variants in the NOD2 gene (IBD1) have been associated with susceptibility to Crohns disease (CD). However, in a previous genome wide scan of 158 Canadian affected sibling pairs linkage to the IBD1 locus was not detected(1). To determine whether NOD2 mutation is etiologically relevant in this CD population, the frequency and phenotypic consequences of the three major CD-associated NOD2 variants, R702W, G908R and 1007fs, were studied in 507 (238 familial and 269 sporadic) unrelated CD patients. At least one of the three NOD2 variants studied was present in 31.5% of the CD patients compared to 20% of unrelated controls. The frequency of these variant alleles was similar in familial versus sporadic cases. No significant association was found between the presence of a NOD2 variant and age of diagnosis, gender, smoking status, ethnicity (non Jewish Caucasian versus Ashkenazi Jewish) or disease severity. However, the R702W variant occurred more frequently in non-Jewish versus Jewish CD patients (10.2% vs 4.5%, $p=0.005$), whereas the G908R variant was more common in Jewish patients (6.8% vs 3.8%, $p=0.04$). A significant positive association was also detected between the presence of NOD2 variant(s) and distal ileal involvement ($p=0.001$), while NOD2 variant(s) were negatively associated with involvement of the colon ($p=0.002$). These data thus reveal a significant influence of NOD2 mutation on the site of bowel involvement in CD and indicate the frequency of NOD2 variants to be similar in Canadian relative to other reported CD populations. The data also reveal NOD2 variants occur more frequently in the controls studied here than reported in other studies, an observation which may account for the failure to detect the IBD1 locus in our genome wide scan. (1) Rioux, JD, et al. *Am J Hum Genet* 2000; 66:1863.

NOTCH4 gene haplotypes are associated with schizophrenia in African-Americans. X. Luo¹, T.A. Klempan², J. Lappalainen¹, R.A. Rosenheck¹, D.S. Charney³, J. Erdos¹, D.P. van Kammen⁴, H.R. Kranzler⁵, J.L. Kennedy², J. Gelernter¹. 1) Dept Psychiatry, Yale Univ Sch Medicine, New Haven, CT; and VA CT Healthcare Center, West Haven, CT; 2) Neurogenetics, CAMH, Toronto, ON, Canada; 3) NIMH, Bethesda, MD; 4) J&J Pharm R&D, Raritan, NJ; and Univ Penn, Philadelphia, PA; 5) Univ Conn Sch of Medicine, ARC, Dept Psychiatry, Farmington, CT.

As an important locus within the HLA region, NOTCH4 is both a positional and a functional candidate for influencing susceptibility to schizophrenia. A recent study (Wei et al., 2000) reported associations between polymorphisms at NOTCH4 and schizophrenia in trios of British descent. Several subsequent studies failed to replicate the association. To evaluate a possible association in African-American (AA) and European-American (EA) subjects, two SNPs at the NOTCH4 locus were genotyped in 123 AA schizophrenics, 223 EA schizophrenics, 87 AA normal controls and 212 EA normal controls. The specific polymorphisms studied were "SNP1" (-1725T/G) and "SNP2" (-25T/C). Comparisons of allele and haplotype frequencies between cases and controls were performed with the Chi-square test or Fisher's Exact Test. Linkage disequilibrium (LD) between these two SNPs was evaluated with 3LOCUS (Long et al., 1995). We found that haplotype -1725G/-25T associates with schizophrenia ($p=0.0012$) in the AA sample. There was no such association in the EA sample. Alleles -1725T and -25C are in positive LD in AA schizophrenics, EA normal controls and EA schizophrenics. We conclude that the haplotype -1725G/-25T at the NOTCH4 locus, which results from SNPs of NOTCH4 that are in LD, may increase susceptibility to schizophrenia in AAs, and that any effect of this locus on risk for schizophrenia is population-specific. (1. Wei J, et al. Nat Genet 2000; 25: 376-377; 2. Long JC, et al. Am J Hum Genet 1995; 56: 799-810).

Deficient alleles (S and Z) of alpha-1 antitrypsin gene among patients with intracranial aneurysm in Japanese population. *T. YONEYAMA*^{1,2}, *H. ONDA*², *H. KASUYA*², *T. NAKAJIMA*¹, *T. HORI*², *I. INOUE*¹. 1) GENETIC DIAGNOSIS, IMS UNIV.OF TOKYO, TOKYO, JAPAN; 2) NEUROSURGERY, TOKYO WOMEN'S MEDICAL UNIV. , TOKYO, JAPAN.

Background and Purpose- We have already performed a genome-wide linkage study of intracranial aneurysm (IA) and detected positive evidence of linkage at chromosome 5, 7 and 14. The follow-up investigations were continued to identify the susceptibilities of IA from the three loci. Previously, it was reported that possible association could exist between the formation of intracranial aneurysm (IA) and deficiency alleles (S and Z) of alpha-1 antitrypsin (AAT) gene, which encodes a major circulating protease inhibitor. Because AAT is locating chromosome 14 near D14S280, the gene was extensively screened in Japanese IA. **Methods-** Genomic DNAs were obtained from 260 IA patients and 293 controls. The seven allelic variants including S and Z alleles were genotyped by direct sequencing. The haplotype in phase-unknown samples was constructed with the expectation-maximization method. Differences in allelic and haplotype frequencies between patients and controls were evaluated by chi-square statistics. **Results-** Significant differences in allelic frequencies were not observed at all seven variants between IA patients and controls. **Conclusions-** We could not detect S and Z alleles of AAT gene in our Japanese samples. The other allelic variants that result in AAT deficiency were not associated with the development of IA. Taken together, AAT may not constitute the susceptibility of IA among Japanese. Possible involvement of other candidate genes for IA could be discussed.

Sequence alterations in the lipoprotein lipase gene in type 2 diabetes patients with hypertriglyceridemia. P.M.K.

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The purpose of this study was to investigate the role of LPL gene variants on patients with hypertriglyceridemic type 2 diabetes with a view to detect genetic factors contributing to most patients with type 2 diabetes. 200 type 2 diabetes patients and 198 healthy control subjects were recruited for the study. We screened for sequence changes in LPL with polymerase chain reaction, single-strand conformation polymorphism, restriction analysis and direct DNA sequencing. LPL mass and activity in post-heparin plasma and in in vitro expression were investigated. Four missense mutations were detected in patients but not controls. Ser447Ter was detected in both patients and controls with no significant difference in the frequencies. The four missense mutations were located in the highly conserved exon 3, 5, and 6 regions and in highly conserved amino acid sites. They led to reduced LPL mass and enzyme activities in post-heparin plasma and in in vitro expression. These results indicated that the 4 missense mutations lead to LPL deficiency and subsequent hypertriglyceridemia. Based on our study and published data, a putative pathogenic pathway was suggested: LPL enzyme deficiency causes elevated plasma triglyceride level and subsequent insulin resistance.

CCR2 and CCR5 gene polymorphisms and susceptibility to sarcoidosis. *T.A. Wischmeijer¹, C. Bombieri¹, C. Agostini², G.P. Semenzato², M. Luisetti³, P.F. Pignatti¹.* 1) Sec. Biology & Genetics, DMIBG, University of Verona; 2) Clin. Immunology, Dpt. of Clinical and Experimental Medicine, University of Padova; 3) Inst. Respiratory Diseases, IRCCS, S.Matteo, University of Pavia; Italy.

Sarcoidosis is a multisystem granulomatous disorder of unknown etiology, that is characterised by high activity of macrophages and CD4+ helper T cells, and by the formation of noncaseating granulomas in a variety of organs, predominantly the lung. A number of chemokines are produced by alveolar cells in the course of inflammatory reactions of sarcoidosis. Interplay between chemokines and their receptors is considered to be crucial for transmigration of lymphocytes and monocytes from the circulation to the bronchoalveolar space. Polymorphisms resulting in a non functional product expression were described in two chemokine receptor genes: a Val to Ile substitution in the CCR2 gene (CCR2-V64I) and a 32 bp deletion in the CCR5 gene (CCR5-del32). Different distribution of these alleles between sarcoidosis patients and controls were described for both the polymorphisms in the Czech population (*Am J Resp Crit Care Med* 162, 1000; 2000) and for CCR2-V64I in the Japanese populations (*Am J Resp Crit Care Med* 159, 2021, 1999). On the basis of these findings a role in disease susceptibility for CCR5 gene polymorphism and a protective role for CCR2-64I allele were suggested. We performed a case-control association study in 75 sarcoidosis patients and 86 control subjects in order to elucidate the role of these polymorphisms in the Italian population. CCR5-del32 allele was present in 10/150 (6.7%) chromosomes of sarcoidosis patients and in 8/172 (4.7%) of controls (NS). CCR2-64I allele was present in 13/126 (10.3%) chromosomes of sarcoidosis patients and in 17/164 (10.4%) of controls (NS). In contrast with the studies on the Czech and Japanese populations, our results show that in an Italian population sample, CCR2-V64I and CCR5-del32 polymorphisms did not seem to play a role in the pathogenesis of sarcoidosis.

DNA sequence variants in optineurin in patients with primary open angle glaucoma and low tension glaucoma.

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Glaucoma is an important cause of blindness worldwide. Of the various forms of glaucoma, adult primary open angle glaucoma (adult POAG) is most common. Typically adult POAG is associated with an elevation of intraocular pressure and a characteristic degeneration of the optic nerve. Patients may have deterioration of the optic nerve without an increase in intraocular pressure, and this is called 'low tension' glaucoma (LTG). Recently, mutations in a novel gene, optineurin, have been described in patients with LTG. The purpose of this study is to determine the frequency of mutations in optineurin in patients with adult POAG and in patients with LTG. Using dHPLC and direct sequencing, we screened all 13 coding exons of the optineurin gene in 86 patients with adult POAG, 38 patients with LTG, and 80 ethnically and age-matched control patients for DNA sequence changes. Two amino acid changes were identified: Met98Lys in 8/86 adult POAG probands, 8/80 control patients and in 6/38 LTG patients; and, E50K in one LTG patient. For the eight POAG individuals with the Met98Lys variant, all available pedigree members were screened. We found that 14 of the 25 affected individuals (56%) and 9 of the 13 unaffected individuals (59%) carried the mutation. Consistent segregation of the mutation with the disease was not demonstrated in any of the eight families. Our results indicate that DNA sequence variants in optineurin are not associated with disease in patients with adult POAG. Alterations of the optineurin gene may be a rare cause of low tension glaucoma. Collectively these results suggest that low tension glaucoma is a genetically distinct disease that is not a major component of the phenotypic spectrum of adult POAG.

CANDIDATE GENE SCREENING OF THE VCFS DELETED REGION FOR MUTATIONS ASSOCIATED WITH SCHIZOPHRENIA. *N.M. Williams¹, N. Norton¹, A. Preece¹, G. Spurlock¹, H. Williams¹, S. Zammit¹, K.C. Murphy², M.C. O'Donovan¹, M.J. Owen¹.* 1) Department of Psychological Medicine, University of Wales College of Medicine, Cardiff, UK; 2) Department of Psychiatry, Royal College of Surgeons, Dublin, Eire.

The df1 murine model of VCFS (del 22q11) displays phenotypic characteristics considered by some to model certain neuro-psychological features of schizophrenia. The df1 deletion spans 22 genes with human orthologs in the 22q11 (ES2, Gscl, Ctp, Vpreb2, Dgcr6, Prodh, ym24d07, Op53c05, Ranbp1, Htf9C, Vo59c07, T10, Arvcf, Comt, TrxR2, Wdr14, Tbx1, Gp1bb, Pnutl, TmVCF, Cdc45l, Ufd1l). In light of the association between VCFS and psychosis, and also the neuro-psychological findings in the df1 mice, these 22 genes are positional candidate genes for schizophrenia. We have examined whether variation within any of these genes confers susceptibility to schizophrenia. We screened the exons and flanking intronic sequence of each gene for sequence variation in 14 individuals with DSM-IV. All polymorphisms identified were genotyped using DNA pooling in a sample of 368 schizophrenics and matched controls. Evidence for association has been found for 1 of the df1 genes, a finding that has been confirmed by individual genotyping of the 368 subjects. We are currently examining this finding in our extended sample of more than 700 schizophrenic cases and controls, in family based association samples, and in VCFS subjects with and without psychosis.

A polymorphism in the C-reactive protein (CRP) promoter is associated with increased prevalence of type 2 diabetes mellitus in Pima Indians. *J.K. Wolford, R.L. Hanson, J.D. Gruber, B. Vozarova, V.M. Ossowski, C. Bogardus.* NIDDK/PECRB, NIH, Phoenix, AZ.

Chronic activation of the immune system may play an etiologic role in the development of type 2 diabetes mellitus (T2DM). Results from recent longitudinal studies report that higher baseline levels of C-reactive protein (CRP), a humoral marker of inflammation, predict the development of T2DM independent of adiposity in Caucasians. In addition, plasma CRP levels are substantially increased in diabetic subjects. It is known that plasma CRP levels are heritable and interestingly, the CRP gene is located on chromosome 1q21 where we have reported linkage to T2DM in Pima Indians. We therefore evaluated CRP as a candidate T2DM susceptibility gene. We screened the CRP locus, including 2 kb of flanking sequence in both the 5' and 3' directions, to identify allelic variants and test them for association with T2DM in Pimas. We also investigated the adjacent amyloid P component, serum (APCS) gene, which is located within 124 kb of CRP. APCS is structurally similar to CRP and increased serum protein levels are also increased in subjects with diabetes. We identified 27 informative polymorphisms, including 26 single nucleotide polymorphisms (SNPs) and 1 insertion/deletion, which were assigned to 7 linkage disequilibrium clusters. We genotyped representative SNPs in ~1300 Pima samples and found one C/T polymorphism (SNP-133552) in the CRP promoter that was significantly associated with T2DM ($P=0.006$). In addition, the variant TT genotype was associated with lower insulin secretory function as measured by 25 g IVGTT ($p=0.05$, adjusted for age, sex, percent body fat, and insulin sensitivity), indicating that CRP might increase the risk of T2DM by decreasing insulin secretory function. These findings suggest that variation within the CRP locus may play a role in diabetes susceptibility in Pima Indians.

CORRELATION OF CLINICAL FEATURES OF WILLIAMS SYNDROME WITH PARENT OF ORIGIN TO IDENTIFY THE POSSIBLE ROLE OF IMPRINTING. *H.K. Wijesuriya¹, H.J. Antoine¹, G. Mohapathra¹, U. Bellugi², F. Rose², J.R. Korenberg¹.* 1) Med Genet, Cedars-Sinai Med Ctr, UCLA, Los Angeles, CA; 2) Salk Institute, San Diego, CA.

Williams Syndrome (WMS) is a neurodevelopmental disorder with variable phenotypes. Phenotypic variability in WMS subjects could be due to differences in the size of deletion, however approximately 95% of WMS subjects carry a deletion of the same 18 known genes located on 7q11.23. We have considered the hypothesis that phenotypic variability could be due to the alteration of gene expression due to the parent of origin of the remaining alleles. Genomic imprinting is defined as the total or partial inactivation of an allele as a consequence of having passed through gametogenesis in one parent. To identify the possible role of imprinting in WMS clinical phenotypes we determined the parent of origin of 62 subjects (47 Trios & 15 single parent families) with confirmed elastin deletion status. To do this we genotyped patients and available parents using 11 microsatellite markers covering the deletion and flanking regions. Of the 62 families tested 61 cases were informative, of which 36 were maternally derived and 25 were paternally derived. This is consistent with increased number of maternal over paternal deletions found in previous data. A deletion was detected at D7S489U locus in 33.3% of cases, D7S2476 in 52.4% cases, ELN1 in 61.1% of cases, and D7S1870 in 38.7% of cases. A deletion was detected at locus D7S489L (larger deletion) in 2 of 59 cases (3.4%), and 19 of 59 cases (32.2%) were not deleted (normal) at the D7S489L locus. A X² analysis was used to assess the influence of parent of origin status on historical, clinical and physical criteria for WMS (Am. Acad. of Ped., 2001) revealed no significant correlation between the degree of clinical phenotypes and the parent of origin. However significant trends were seen in hypertension ($p=0.073$), joint limitation ($p=0.134$), strong attraction to music ($p=0.066$), language delay ($p=0.093$) and short nose ($p=0.109$). The present data support a trend for the presence of imprinted genes in the deleted regions that may contribute to some of the clinical findings of WMS. A larger sample size may be necessary to obtain significance.

Carboxypeptidase A5: an imprinted member of the CPA gene family. *L. Bentley*¹, *K. Nakabayashi*², *Z. Montamedi*¹, *M. Preece*³, *S. Scherer*², *P. Stanier*¹, *G.E. Moore*¹. 1) Maternal and Fetal Medicine, IRDB, London, United Kingdom; 2) Hospital for Sick Children, Toronto Canada; 3) Institute of Child Health, University College London, London, United Kingdom.

Identification of segmental maternal uniparental disomy (mUPD) of 7q31 to the telomere in a Silver-Russell (SRS) patient emphasises this domain as a candidate region for the syndrome (1). Since mUPD7 has been found in approximately 10% of SRS patients, the causative gene is expected to behave in an imprinted way. The human homologue (*MEST*) of mesoderm-specific transcript (*Mest*) was the first imprinted gene to be identified on chromosome 7. It has been localised to chromosome 7q32 and is flanked by biallelically expressed genes, *TSGA14* (testis-specific protein A14) in a centromeric direction and *COPG2* (nonclathrin coat protein) in a telomeric direction (2). Subsequently, *CIT1* (*COPG2* antisense) and *MESTIT1* (*MEST* intronic transcript 1) at 7q32 have been described with imprinting effects (3). Since biallelically expressed genes can occur within a cluster of imprinted genes, the search for further imprinted genes was continued beyond *TSGA14* to the carboxypeptidase (*CPA*) group of genes. At least three transcripts of *CPA5* have been identified by RT-PCR, two of which are expressed in a range of fetal tissues. Single nucleotide imprinting analysis of these isoforms in fetal tissues has revealed tissue specific monoallelic expression. We also report the results of a mutation screening on non mUPD7 SRS patient DNAs. 1. Hannula et al. 2001 Am. J. Hum. Genet. 68:247-253 2. Yamada et al. 2002 Gene. 288:57-63 3. Nakabayashi et al. 2002 Hum. Mol. Genet. In press.

Association study of neurotrophic tyrosine kinase receptor type 2 (NTRK2) and childhood-onset depression. *J. Adams*¹, *K. Wigg*¹, *N. King*², *M. Kovacs*³, *J.L. Kennedy*², *C.L. Barr*^{1,4}. 1) Molecular & Cellular Division, The Toronto Western Hospital, Toronto, ON, Canada; 2) Centre for Addiction and Mental Health, Toronto, ON, Canada; 3) University of Pittsburgh, Pittsburgh, PA; 4) The Hospital for Sick Children, Toronto, ON, Canada.

Childhood-onset depression (COD) is a particularly serious form of affective illness. Extensive evidence suggests that depressive disorders are familial and twin studies suggest a substantial genetic component with increased genetic risk for relatives of childhood-onset probands. Several lines of evidence suggest a relationship of depression to neurotrophic factors. Brain derived neurotrophic factor (BDNF) is believed to be a key component of neural plasticity as it promotes neuronal survival and differentiation during development and encourages neuron growth during adulthood. Moreover, stress has been shown to reduce BDNF expression whereas chronic anti-depressant treatment increases expression. Also, administration of BDNF in both cellular and animal models has anti-depressant like effects. BDNF acts through its high-affinity receptor tyrosine kinase B (Trkb) which is encoded by the gene NTRK2. We hypothesize that DNA variations in NTRK2 relate to an individual's vulnerability to developing COD. We tested for association of NTRK2 with COD by comparing the distribution of a 2 allele polymorphism in NTRK2 in 104 cases that met DSM-IV criteria for a major depressive disorder before the age of 14 to a representative control sample balanced for ethnicity and gender. We found that the allele frequency between the two groups did not differ (chi square = 1.184, 1 df, p=0.277) and that the COD group showed no significant difference in genotype distribution compared to controls (chi square = 3.014, 2 df, p=0.222). Additional markers in this gene are currently under investigation.

***GABRA2* is associated with alcohol dependence and brain oscillations in a large family-based sample from the Collaborative Study on the Genetics of Alcoholism.** *H.J. Edenberg*¹, *D.M. Dick*¹, *B. Porjesz*², *X. Xuei*¹, *T. Foroud*¹, *H. Begleiter*² and *COGA Collaborators*. 1) Indiana Univ Sch Medicine, Indianapolis, IN; 2) SUNY HSC, Brooklyn, NY.

Linkage analyses using microsatellite markers identified a region of chromosome 4p that contained genes affecting the risk for alcoholism and affecting brain oscillations in the beta frequency (13-28 Hz). Within this region is a cluster of genes encoding GABA(A) receptors; these are excellent candidates for affecting brain oscillations through their role in mediating fast synaptic inhibition, and for affecting risk of alcoholism. We pursued these candidate genes by genotyping and analyzing multiple SNPs in *GABRA2*, *GABRA4*, and *GABRB1* in more than 2200 individuals from 262 families containing multiple alcoholics systematically ascertained by the Collaborative Study on the Genetics of Alcoholism. Linkage disequilibrium (LD) between markers within each gene was high; LD between genes was lower. Analyses of these SNPs provide evidence that variations in the *GABRA2* gene, encoding the alpha subunit of the GABA(A) receptor, affect brain oscillations. LD analyses performed using the Pedigree Disequilibrium Test provide strong evidence that the same gene, *GABRA2*, affects the risk for alcoholism, defined as DSM-IV alcohol dependence. SNPs throughout the *GABRA2* gene were significantly associated with alcohol dependence. The analyzed SNPs do not affect the amino acid sequence of the receptor subunit, but might be related to subtle differences in gene expression. The convergence of evidence from different analyses and phenotypes on this gene provides strong evidence that *GABRA2* is a key gene affecting the risk for alcoholism, and provides a plausible biological hypothesis that its effect is mediated by alterations in brain oscillations that affect the level of excitation.

A polymorphism in ENPP-1 that is associated with ectopic ossification is also associated with diabetic end-stage renal disease. *J.P. Bochenski, P.P. Wolkow, L.H. Canani, A. Smiles, M.G. Pezzolesi, J.H. Warram, A.S. Krolewski.*
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A polymorphism in exon 4 of the ecto-nucleotide pyrophosphatase/phosphodiesterase 1 (ENPP-1) gene resulting in an amino acid change (K@Q) is associated with insulin resistance and recently has been shown to be associated with end-stage renal disease (ESRD) in patients with diabetes. A different polymorphism in the ENPP-1 gene, a T insertion/deletion in intron 20 (T ins/del) has been shown to be associated with the ossification of the posterior longitudinal ligament of the spine. This suggested the hypothesis that mechanisms involved in calcification of connective tissue may also be involved in glomerulosclerosis in kidneys. We examined this hypothesis in a case-control study of 144 cases with diabetic ESRD and 281 controls with normoalbuminuria despite more than 15 years duration of type 1 diabetes. DNA from cases and controls, which had already been genotyped for the exon 4 polymorphism, was genotyped for the T ins/del in intron 20. The deletion was significantly more frequent in ESRD cases than controls ($p=0.02$) and the odds ratio for ESRD among carriers of the deletion was 1.7 ($p=0.01$). This association is almost identical in magnitude to that shown previously for carriers of the Q allele at exon 4. However, linkage disequilibrium between the polymorphisms in exon 4 and intron 20 is only modest ($D=0.55$). Two other polymorphisms have been identified and in preliminary analyses the odds ratios for ESRD range from 2 to 4. The critical haplotype for ESRD is being searched for using a family-based study design (TDT). Our data indicate that sequence differences in the ENPP-1 locus contribute to genetic susceptibility to ESRD in diabetes. Pathways involved in ectopic ossification may underlie glomerulosclerosis leading to ESRD in diabetes.

The expression pattern of 119 genes from the region of 18q21-22 that has shown linkage to bipolar disorder. *H. Chen*¹, *Y. Huo*¹, *N. Wang*¹, *D.F. MacKinnon*¹, *J.B. Potash*¹, *P. Sklar*³, *R. DePaulo, Jr.*¹, *C.A. Ross*^{1,2}, *M.G. McInnis*¹.
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Linkage evidence of a locus on chromosome 18q21-22 to bipolar disorder has led to the effort to identify novel genes expressed in brain from this region for mutation study. We selected all 119 genes (35 known and 84 predicted) in the region annotated at the Celera databases, and created a gene array for expression analysis. To test the genes whether they are expressed in brain, we amplified one exon of each gene by PCR and made dot blot filters using 20 ng of PCR products for each gene. The filters in duplicate were then hybridized with ³²P labeled probes derived from 2 mg of normal brain polyA+ RNA (Clontech). We used a PhosphorImager scanner (Molecular Dynamics) to scan the hybridization images, and the ImageQuant software (Molecular Dynamics) to analyze data. In this study, we identified 22 known and 2 novel genes expressed in brain with various levels. We observed that 3 genes showed expression reduction of at least two fold when the array was hybridized with a probe derived from RNA isolated from a postmortem brain of bipolar disorder. This observation is being tested on additional postmortem brain samples of bipolar disorder, and being verified with RT-PCR amplification. The fact that few predicted genes were detected on the array then lead us to test by use of Northern blot analysis the genes for expression. We selected 20 such genes and performed expression analysis using a commercial Northern blot (Clontech #7760-1). None of the genes tested showed detectable hybridization signal. It is possible that these genes are expressed at very low levels hardly to be detected by Northern blot assay, or that a substantial number of predicted genes are not expressed in the tissues examined, or false prediction.

No evidence for linkage or linkage disequilibrium to nine circadian clock genes in bipolar disorder. *C.M. Nievergelt, D.F. Kripke, N.J. Schork, J.R. Kelsoe.* Department of Psychiatry, University of California, San Diego, CA.

Bipolar disease is suspected to be associated with malfunctions of the circadian system, which regulates individual circadian rhythms and enables the adaptation to a daily 24-hour cycle. Several human clock genes have been identified and localized and a comparison with linkage hotspots for bipolar disorder has revealed close correspondences. Linkage and linkage disequilibrium (LD) of nine clock genes (cryptochrome 1 and 2, period 1, 2, and 3, clock, *bmal1*, casein kinase 1e, and D box binding protein) to bipolar disorder were analyzed here.

Linkage analysis using 18 microsatellites in 52 affected families showed suggestive evidence for linkage to one (CK1e) of the nine genes. Because of the complex nature of bipolar disease, heterogeneity tests and multi-locus analysis were performed. No additional evidence for linkage under single locus or multi-gene models was found.

Because of its close location to a linkage hotspot, *cry1* was further analyzed by screening for functional mutations in 25 bipolar subjects from families with suggestive linkage to this gene. 13kb of the 102kb gene were sequenced and 17 variations (16 SNPs and a insertion) were identified. Two SNPs were located in the promotor region. The only coding sequence variant identified was found to be synonymous.

Multiple polymorphic SNPs for each of the nine clock genes, identified by sequencing for cryptochrome 1 and selected from databases for the other eight genes, were genotyped in 185 affected parent proband triads. LD analyses using TDT tests for single SNPs as well as haplotype analyses did not show evidence for association to bipolar disease.

In summary, we found no significant evidence for association or linkage of any of the clock genes examined. However, we have examined only a subset of putative clock genes. Further extension of this study would include examination of other clock genes and of subforms of the bipolar phenotype that have abnormalities of circadian rhythms.

Familial Meniere's disease in Finland. *M. Männikkö*¹, *J. Kotimäki*², *E. Jakkula*¹, *M. Sorri*³, *L. Ala-Kokko*¹. 1) Collagen Research Unit, Biocenter Oulu, and Department of Medical Biochemistry and Molecular Biology; 2) Central Hospital of Kajaani; 3) University Hospital of Oulu, Finland.

Menieres disease (MD) is characterized by fluctuating sensor neural hearing loss, vertigo, and tinnitus. Its etiology still remains unknown and treatment is largely empirical. It is usually defined idiopathic, but suggested to be due to a variety of causes including trauma, viral infections and autoimmune factors. Both autosomal dominant and recessive inheritance has been documented for MD. The incidence in Finland is 43/100 000, and the estimate of hereditary MD is 5-15%. In the present study we have collected 26 Meniere-patients with positive family history from 11 families with 25 non-affected family members, and 42 patients with no family history of the disease. The mode of inheritance for MD in Finland was defined as autosomal dominant with low penetrance and anticipation in two families. The candidate gene approach was used to analyze the COCH and COL11A2 genes for sequence variations. Both genes are expressed in the inner ear with a putative role in MD. Sixty-four Meniere-patients were screened for P51S mutation in the COCH gene causing autosomal dominant non-syndromic progressive sensor neural hearing loss with MD-like symptoms. None of the patients carried this mutation. Conformative sensitive gel electrophoresis analysis (CSGE) and subsequent sequencing of the change containing exons was performed for the coding region and surrounding sequences of the COCH gene. Four polymorphic changes were found, that were also found from the control set from general population. An HLA-allele, Cw7, was reported to be more abundant in MD than in controls suggesting a linkage to HLA-region (Chr 6), where COL11A2 also locates. Mutations in the COL11A2 gene have been reported in syndromic hearing loss associated with collagen related diseases but also in non-syndromic hearing loss, DFNA13. CSGE analysis of the COL11A2 gene was performed. Nineteen polymorphic changes were found with similar incidence than observed in general population. In addition an arginine to proline change and two intronic changes were found that were not observed in general population.

Association of a single nucleotide polymorphism of the DAZL gene with susceptibility to spermatogenic failure.

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Purpose: To investigate the association of DAZL gene with spermatogenic failure. **Materials and methods.** Single-strand conformation polymorphism (SSCP) analysis of exon-containing genomic DNA segments of the DAZL gene was performed in 160 infertile Taiwanese men presenting with severe oligozoospermia and non-obstructive azoospermia. Sequence analysis was used to identify the mutations or polymorphisms in the fragments with aberrant SSCP patterns. All cases also underwent PCR analysis to characterize deletion status of 15 Y-chromosome genes. **Results.** An AG transition at nucleotide 260 (260 A->G) in exon 2, and an AG transition at nucleotide 386 (386 A->G) in exon 3 were identified. The mutation in exon 2 leads to a Thr12Ala change (T12A) of the DAZL protein. There was no difference in allelic frequency in T12A polymorphism between patients with spermatogenic failure and control subjects ($P=0.542$). The mutation in exon 3 is located within the RNA-recognition motif of the DAZL protein and will lead to Thr54Ala change (T54A). The frequencies of T54A allele in patients and control group were 7.39% and 0.86%, respectively ($p=0.0003$). There was no linkage disequilibrium between T12A and T54A alleles. The phenotypes varied significantly in cases with heterozygous T54A polymorphism, ranging from hypospermatogenesis and maturation arrest to Sertoli cell-only syndrome. A combination of DAZ gene deletion and T54A polymorphism did not worsen the phenotype. **Conclusion.** Our findings provide strong evidence for the role of the autosomal DAZL gene in human spermatogenesis. Heterogeneity of phenotypes in patients with T54A variant indicates involvement of complex genetic components in spermatogenic failure.

Microarray Analysis of Postmortem Multiple Sclerosis Cortex. *J. McDonough¹, J. Peterson¹, R. Klinkosz¹, K. Mirnics², B.D. Trapp¹.* 1) Dept Neuroscience, Cleveland Clinic Foundation, Cleveland, OH; 2) University of Pittsburgh School of Medicine, Neurobiology Dept. and Pittarray, Pittsburgh, PA.

Multiple Sclerosis is an inflammatory disease of the CNS marked by loss of myelin, oligodendrocytes, and neurons. MS is a complex disease in which susceptibility is conferred by many genetic loci in combination with environmental factors. It is hypothesized to be at least in part an autoimmune disease in which an immune response is directed against myelin proteins. The cause or causes of MS, however, are unknown. A component of MS relatively overlooked until recently is the pathology involving neurons. There are several mechanisms by which neurons are destroyed in MS and the possibility that MS is a primary neurodegenerative disease with secondary inflammatory white matter lesions has been raised. We are interested in identifying genes in neurons which are involved in the neuropathology associated with MS. In this study we have identified genes differentially expressed in MS cortical lesions vs. control cortex by microarray analysis. We have 27 MS brains from rapid autopsies from the Cleveland Clinic Foundation. Control brains have been obtained from the University of Pittsburgh and are matched for age, sex, postmortem interval, race, and pH. Lesions were identified and staged in postmortem MS brains with antibodies to myelin proteins and microglia. We then isolated mRNA from these lesions and from normal cortex and made probes which were hybridized to either Incyte or Affymetrix human microarrays. We have analyzed four cortical lesions and have identified gene groups which are consistently changed. Myelin protein genes were decreased in all lesions analyzed. The expression of presynaptic genes, genes involved in calcium homeostasis, mitochondrial genes, cytoskeletal element genes, and intracellular signalling genes are also changed in the cortical lesions. More comparisons are needed however to determine which changes are significant and to identify genes that will be candidates for further study.

Linkage of the Dopamine Receptor D1 Gene to Attention-deficit/hyperactivity Disorder. *V.L. Misener¹, P. Luca¹, O. Azeke¹, R. Tannock², W. Roberts³, M. Malone³, R. Schachar², J. Crosbie², A. Ickowicz², J.L. Kennedy⁴, C.L. Barr^{1,2}.*
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Attention-deficit/hyperactivity disorder (ADHD) has a strong genetic component, and genes of the dopamine system are considered primary candidates because medications that are effective in the treatment of this disorder influence the dopamine system. This has been borne out by positive and replicated linkage findings for the dopamine receptor D4 (DRD4) and dopamine transporter (DAT1) genes.

Evidence from both human and animal studies, which includes hyperactivity exhibited by dopamine receptor D1 (DRD1)-knockout mice, suggests that the DRD1 gene is a strong candidate for involvement in ADHD. Here, we tested for linkage of DRD1 to ADHD, by examining the transmission of three bi-allelic DRD1 polymorphisms (D1P.5, D1P.6 and D1.7) in a sample of 157 nuclear families identified through an ADHD proband. Using the transmission disequilibrium test, we observed trends for biased transmission of alleles for each of the three markers individually (*ie.* biased towards transmission of D1P.5 Allele 1, $p=0.122$; D1P.6 Allele 1, $p=0.105$; D1.7 Allele 2, $p=0.146$), and obtained significant evidence for linkage when haplotypes for these markers were analysed. Specifically, among three haplotypes which were sufficiently informative for the analysis in our sample (*ie.* 1.2.1, 2.2.1 and 1.1.2), we observed a bias for transmission of the 1.1.2 haplotype ($p=0.011$) and for non-transmission of the 2.2.1 haplotype ($p=0.022$). These findings support the proposed involvement of the DRD1 gene in ADHD. Since none of the polymorphisms analysed here is predicted to alter DRD1 function, we are currently screening the gene, by sequencing, to identify functional DRD1 variant(s) in our ADHD sample.

Association of ADAM33 with asthma and associated phenotypes in ethnically diverse populations. *T.D. Howard¹, D.A. Meyers¹, E.A. Ampleford¹, S.L. Zheng¹, H. Jongepier², D.S. Postma², E.R. Bleeker¹.* 1) Center for Human Genomics, Wake Forest Univ Sch Med, Winston-Salem, NC; 2) Dept of Pulm, Univ Hospital, Groningen, the Netherlands.

Asthma is a common respiratory disease characterized by intermittent airways obstruction and respiratory symptoms that are caused by acute and chronic bronchial inflammation. A recent study of 483 Caucasian families from the US and UK used a positional cloning approach to identify a candidate region on chromosome 20p for susceptibility to asthma, bronchial hyperresponsiveness (BHR), and total and specific IgE levels (patent number WO 01/78894). Further investigation identified the putative candidate gene as A Disintegrin And Metalloprotease (ADAM) 33. Multiple single nucleotide polymorphisms (SNPs) within ADAM33 were significantly associated with asthma and bronchial hyperresponsiveness (BHR) phenotypes for either the US, UK, or both populations combined. The ADAM family of transmembrane proteins has been implicated in critical functions such as cell-cell and cell-matrix interactions, cell migration, cell adhesion, and signal transduction. One role of ADAM genes studied to date is the shedding of the extracellular portion of specific cytokines and growth factors, leading to soluble forms of these proteins. In an effort to further characterize the contribution of ADAM33 to asthma, we evaluated a subset of the previously described SNPs in four populations: Dutch, US Caucasian, African-American, and US Hispanic. Significant associations were observed with multiple SNPs within ADAM33 and asthma, BHR, and total serum IgE levels in one or more of the four populations. For example, for the SNP labeled Q_2, significant associations were observed with measure of skin test responsiveness in the Dutch ($p=0.01$), BHR in US Caucasians ($p=0.045$), asthma in US African-Americans and Hispanics ($p=0.04$, 03). Further studies of this gene and its role in asthma are needed.

No association of polymorphisms in the cholinergic locus with late-onset Alzheimer's disease. *D. Harold¹, T. Peirce¹, M. Hamshere¹, A. Myers², D. Turic¹, S. Lovestone³, J. Powell³, S. McIlroy⁴, D. Craig⁴, P. Passmore⁴, J. Hardy⁵, A. Goate⁶, M. Liddell¹, M. O'Donovan¹, J. Williams¹, M.J. Owen¹, L. Jones¹.* 1) Dept of Psychological Medicine, UWCM, Cardiff, United Kingdom; 2) Washington University, St Louis, United States; 3) Institute of Psychiatry, London, United Kingdom; 4) Queens University, Belfast, United Kingdom; 5) National Institute on Aging, Bethesda, United States; 6) Dept of Psychiatry, St Louis, United States.

The genes for choline acetyltransferase (CHAT) and the vesicular acetylcholine transporter (VACHT) are strong functional and positional candidates for late-onset Alzheimer's disease (AD). One of the hallmarks of AD is the widespread dysfunction of the basal forebrain cholinergic system. The activity of CHAT has been shown to be reduced by 50-90% in AD patients compared with age-matched controls and is correlated with the depth of dementia. At present, the most effective therapies in treatment of the early stages of AD target this loss of function through the action of cholinesterase inhibitors. In addition, CHAT and VACHT are transcribed from a single locus on chromosome 10, in a region showing strong evidence of linkage to AD. We used DHPLC and sequencing to identify sequence variation in all known exons plus 3kb of putative regulatory sequence 5' to the multiple transcriptional start sites of these genes. We found 17 sequence variants: 16 single nucleotide polymorphisms and one minisatellite. Of the four polymorphisms in the coding exons, three were non-synonymous. A further 12 SNPs were observed in introns, untranslated and regulatory regions. We analysed all of the variants in a UK sample of late-onset AD cases and controls, consisting of 131 cases with probable late-onset AD and 106 age-matched controls sampled from the UK population. We found no significant association with AD. Haplotype analysis revealed some positive two-marker associations with a SNP in intron 9 of CHAT, but this was not replicated in a further sample of 242 AD cases and 235 controls collected in Belfast. We conclude that there is no genetic association between variation in CHAT or VACHT and late-onset AD.

A search for CCG expansion mutations in schizophrenia linkage regions. *J.J. Kleiderlein¹, J.B. Potash¹, L. Monfredo¹, S. Pirzadeh¹, H. Hwang¹, C.A. Ross^{1,2,3}, L.E. DeLisi⁴, R.L. Margolis^{1,3}, S.E. Holmes¹.* 1) Dept of Psychiatry,; 2) Dept of Neuroscience; 3) Program in Cellular and Molecular Medicine, Johns Hopkins Univ Sch Med, Baltimore, MD; 4) Dept of Psychiatry, NYU, New York, NY.

Many investigators have searched for a causal link between CAG expansion mutations and schizophrenia, but there has not been a rigorous study of CCG repeats in this disorder. Fragile X syndrome, a neurodevelopmental disorder with phenotype including psychiatric features, is caused by a large CCG expansion mutation. Schizophrenia is also hypothesized to have a neurodevelopmental basis, and we thus undertook a systematic search for CCG expansion mutations in a set of 65 unrelated individuals with schizophrenia. This is a clinically well-characterized collection of subjects ascertained in the USA and Europe on the basis of having at least two full siblings with the diagnosis of schizophrenia or schizoaffective disorder. We have also examined CAG repeats in this sample (Tsutsumi et al., 2002). We selected linkage regions that have been confirmed in more than one sample set, those with particularly high LOD scores, and regions showing linkage in our sample set (DeLisi et al., 2002). Within these regions (1q22-1q42, 2p11-2q14, 6p25-6p21, 6q14-6q27, 8p23-8p12, 10p14-10p12, 13q31-13q34, 15q13.14-15q13.3, and 22q11.2-22q13) we identified 35 CCG repeats containing 8 or more consecutive triplets. We developed PCR assays including Southern blots to screen for expansion mutations. No expansions were detected in the initial 18 repeats tested. This evidence does not support the hypothesis that a CCG expansion mutation is involved in schizophrenia, but additional studies are underway. DeLisi, L.E. et al., *Am J. Psychiatry* 2002; 159; 803-812. Tsutsumi, T. et al., ASHG 2002.

Positive allelic association of the marker d21s171 with bipolar disorder identifies TRPC7, a calcium channel protein on chromosome 21 at 21q22.3, as being a candidate gene for increasing genetic susceptibility to bipolar and related unipolar affective disorders. A. McQuillin¹, G. Kalsi¹, J. Lawrence¹, C. Smyth¹, D. Curtis², N.J. Bass¹, H.M.D. Gurling¹. 1) Molecular Psychiatry Laboratory, Department of Psychiatry, Royal Free and University College London Medical School, Windeyer Building, 46 Cleveland Street, London W1T 4JF, UK; 2) Department of Psychological Medicine, St Bartholomew's and Royal London School of Medicine and Dentistry, Whitechapel, London E1 1BB, UK.

Linkage analysis of bipolar families from many independent investigators have identified the distal end of the long arm of chromosome 21 as a potential source of genetic susceptibility for bipolar affective disorder (Gurling 1998, Smyth et al 1997). We genotyped 22 genetic markers near the telomere of chromosome 21 at 21q22.3 in 300 cases and 300 ethnically matched controls and found one marker that gave significant evidence for allelic association with bipolar disorder after a Monte Carlo correction for multiple alleles. Flanking markers gave weaker evidence for association and were only significant without correction for multiple alleles. We found evidence for a very high rate of recombination in this region because consistent evidence for linkage disequilibrium was difficult to detect even with adjacent markers expected to show disequilibrium. Cases who inherited the D21S171 marker alleles that were associated with bipolar disorder were selected for direct genomic sequencing of the TRPC7 calcium channel gene. Several polymorphic single nucleotide base pair variants within the gene have been detected and their significance in relation to the genetic susceptibility to bipolar disorder is currently being assessed. Gurling H (1998) Chromosome 21 Workshop. *Psychiatr Genet* 8, 109-113. Smyth C, et al (1997) *Genomics* 39,271-278 The research was funded by Medical Research Council grant G9623693N and by a research lectureship from the Priory Hospital, London to Dr. J Lawrence and by the Neuroscience Research Charitable Trust. We would also like to acknowledge the help of Hamish Scott and Stylianos Antonarakis for providing unpublished mapping information and primer sequences.

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Allelic association study investigating GABRA3 and markers in G6PD region in a UK bipolar case-control sample. *G. Kalsi, A. Mcquillin, D. Curtis, N. Bass, H.M. Gurling and Molecular Psychiatry.* Psychiatry & Behav Sciences, Windeyer Inst of Med Sciences, London, UK.

An excess of affected females and a deficiency of male-to-male transmission has led to the proposal of an X-linked form of bipolar illness. Several studies have highlighted a possible link between Glucose-6-Phosphate Dehydrogenase (G6PD) on Xq28 and bipolar illness. The $\alpha 3$ subunit of the gamma amino butyric acid receptor gene (GABRA3) has received attention on the basis of preclinical and pharmacological studies, which suggest that a dysfunction in brain GABAergic system activity could contribute to manic depression. Thus, the $\alpha 3$ subunit localised on Xq28 represents a plausible candidate gene. Previous linkage and association studies have shown mixed results. We used microsatellite markers for GABRA3, G6PD and markers flanking this gene to test for allelic association in a stringently screened sample of 306 UK bipolar affective patients of English, Irish, Welsh or Scottish ancestry and compared allele frequencies in 310 ethnically matched normal controls. The data was analysed using the CLUMP program. Results showed statistically significant evidence in favour of a putative locus in G6PD region. The G6PD-Med polymorphism produced $p=0.005$. However, tests with GABRA3 showed lack of evidence in favour of a possible pathogenic role in our sample.

Evidence for linkage but not association to the GABRB3 region of chromosome 15 in a subset of autistic disorder (AutD) families characterized by an increase in restricted and repetitive behaviors. *M.M. Menold¹, Y. Shao¹, S.J. Kim¹, C.M. Wolpert¹, S.L. Donnelly¹, S.A. Ravan², R.K. Abramson², H.H. Wright², G.R. Delong¹, M.L. Cuccaro¹, M.A. Pericak-Vance¹, J.R. Gilbert¹.* 1) Ctr for Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) WS Hall Psychiatric Institute, Univ.of S. Carolina, Columbia, SC.

Autistic disorder (AutD) is a complex neurodevelopmental disorder with a strong genetic component. While numerous susceptibility genes probably exist for AutD, it has been difficult to detect these genes by linkage or association since each gene is presumed to contribute just a small part to the etiology of the disease. Identification of the genes is made even more complicated because of the clinical heterogeneity present in data sets. Ordered subset analysis (OSA) is a method that can be used to identify a particular set of families that are contributing to linkage or association at a given locus. Using this technique, Shao et al. (this meeting) demonstrated significantly increased LOD scores in the region of the GABRB3 gene by using a factor derived from repetitive behaviors and stereotyped patterns as the covariate for OSA. This analysis strengthened previous results of linkage and association at this locus for AutD.

Using the 23 families (107 individuals) that were identified by OSA as being a homogeneous subset of the AutD data set, we analyzed 9 single nucleotide polymorphisms (SNPs) within the GABRB3 gene and in the region located beyond the 3' end of the gene. Eight of the nine SNPs showed positive linkage with a peak LOD score of 1.673 for SNP GABRB3-3PR1 located 3.6 kb from the end of the gene. Association analysis was not significant for any of the SNPs using either the Pedigree Disequilibrium Test (PDT) or Transmit. While this result supports evidence for an autism gene in the GABRB3 region of chromosome 15, AutD does not appear to be associated with a specific SNP haplotype, and may instead be due to multiple mutation events in this region.

Transmission of SYNAPSIN3 gene variants in schizophrenia families. *T.A. Klempan¹, T. Armstrong¹, N. King¹, C. Pato², M. Pato², M.-H. Azevedo², F. Macciardi¹, J.L. Kennedy¹.* 1) Neurogenetics, CAMH, Toronto, ON, Canada; 2) Department of Psychiatry, University of Coimbra, Portugal.

The synapsins comprise a family of exocytotic vesicle membrane proteins that participate in vesicle mobilization, axon formation, and neural plasticity. The association of synapsins with actin microfilaments following phosphorylation serves to control the recruitment of vesicles, in turn regulating the kinetics of neurotransmission and synaptic strength. The synapsins are highly expressed in brain and several reports have revealed reduced expression of synapsins in the brains of schizophrenic individuals. The SYNAPSIN3 gene has been localized to chromosome 22q13, within 5 cM of D22S278, a dinucleotide repeat marker previously associated with schizophrenia. The possible role of SYNAPSIN3 in the pathogenesis of schizophrenia was investigated through analysis of two substitution polymorphisms within the promoter region (-631C/G and -196G/A) and a dinucleotide repeat (D22S280) within intron 5 of the gene. These markers were studied in a combined sample of 228 small nuclear schizophrenia families, 123 families of variable ethnicity and 105 families from mainland Portugal and the Azores islands. Transmission of alleles to affected individuals was examined using the family-based association test (FBAT). No significant differences in inheritance of these alleles was observed in these pedigrees using FBAT (multi-allelic, dominant model: -631C/G chi-squared=0.518, $p=0.772(2df)$; -196G/A chi-squared=0.246, $p=0.884(2df)$; D22S280 chi-squared=4.591, $p=0.710(7df)$). Comparison of the age at onset for affected individuals shows an effect of homozygosity for the 631G allele on early age at onset ($F=5.14$, $p=0.026$). These findings suggest that while alleles of SYNAPSIN3 are unlikely to act as major susceptibility factor for schizophrenia, they may influence the timing of symptom onset.

Program Nr: 1868 from 2002 ASHG Annual Meeting

Quantitative RT-PCR Analysis of Gene Expression Alterations in Selective Brain Regions of Patients with Mood Disorders. *J. Li¹, P.V. Choudary², S.J. Evans³, M.P. Vawter⁴, H. Tomita⁴, J. Meador-Woodruff³, E.G. Jones², W.E. Bunney⁴, S.J. Watson³, H. Akil³, R.M. Myers¹.* 1) Stanford Human Genome Center, Palo Alto, CA; 2) Center for Neuroscience, Univ. California, Davis, CA; 3) Mental Health Research Institute, Univ. Michigan, Ann Arbor, MI; 4) Department of Psychiatry, Univ. California, Irvine, CA.

We compared transcript levels for several hundred genes in *post-mortem* brain tissues between patients and controls in an effort to identify gene-expression alterations associated with mood disorders. We isolated total RNA from specific brain regions implicated in mood disorders, synthesized first-strand cDNA, and performed SYBR Green-based realtime-PCR experiments to quantitate transcript levels for each gene relative to a panel of reference genes. So far we have analyzed anterior cingulate cortex, dorsolateral prefrontal cortex and cerebellum from nine Bipolar patients, eleven Major Depressive Disorder (MDD) patients and 20 control individuals. The genes included in this study are approximately 150 carefully selected candidate genes covering a variety of functional categories, additional genes previously reported in literature as putatively associated with suicide, Bipolar and/or MDD, as well as genes that appeared to be up- or down-regulated in our cases as compared to controls according to our Affymetrix microarray analysis (reported elsewhere). This presentation will describe results of our quantitative RT-PCR analysis, as well as our methods for achieving high sensitivity and high throughput, while ensuring accuracy. *Supported by: The Pritzker Neuropsychiatric Disorders Research Consortium, Pritzker Family Philanthropic Fund and NIH CONTE Center grant #L99MH60398.*

Mutation analysis for candidate genes in Japanese autistic population. T. Yamagata, H. Li, M. Mori, K. Suwa, M.Y. Momoi. Department of Pediatrics, Jichi Medical School, Tochigi, Japan.

Mutation analysis for candidate genes in Japanese autistic population. T. Yamagata, H. Li, M. Mori, K. Suwa, and M. Y. Momoi. Department of Pediatrics, Jichi Medical School, Tochigi, Japan We are analyzing several candidate genes for possible causative mutations among Japanese autistic children. Patients diagnosed with pervasive developmental disorder or autism according to the criteria of DSM-IV were enrolled in this study after the informed consents by their parents. Up to one hundred autistic patients were enrolled. We report the results of mutation analyses of methylation-related genes, such as *MeCP2*, *MBD1*, *MBD2* and *MBD3*, and the genes located on 7q such as *FOXP2* and serotonin receptor gene (*HTR5A*). All exons and promoter regions of these genes were amplified by PCR and the mutation was detected by DHPLC, and subsequently by direct sequencing. We found two base substitutions in *MBD1* that were not found in 150 control DNA. One was G to A change in the first base of exon 2 (5UTR) in one male patient. The other was C805T that introduced R269C amino acid change in another male patient. The C805T change was also detected in the patients father with some phenotype of autism and in a normal sister. We also found repeat length polymorphism of (GGC)₄ to 5 and (GGGGCC)₂ to 3 in *MBD2*, and (CAA)₅ to 4 in *FOXP2* in some patients, however, we did not detect the difference in the frequency between patients and controls. The significance of these base substitutions with amino acid change in patients and some of their family members is not known, however, the absence of these SNPs among normal populations suggested the relevance to the disease. We also found that methylation-related genes were highly polymorphic, that also suggested the possible involvement of SNPs of these genes in the pathology of autism.

Identification of susceptible gene for the ossification of the posterior longitudinal ligament of the spine from the linkage region on chromosome 21. *T. TANAKA¹, K. IKARI², K. FURUSHIMA¹, S. TOH¹, S. HARATA¹, T.*

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Ossification of the posterior longitudinal ligament of the spine (OPLL) is a subset of bone former disease, characterized by ectopic ossification in the spinal ligaments by an endochondral process. OPLL is a common disorder among Japanese and other Asian populations constituting the leading cause of spinal myelopathies. The incidence of this disorder in Japan is reported to be 1.9 %-4.3 %. Although multiple etiologies for OPLL need to be considered because of the late-onset nature of the disease, it is well recognized that a certain fraction of this common disease is genetically determined. We have performed a genome-wide linkage study with 126 affected sib pairs using GENEHUNTER and SIBPAL programs to identify genetic loci related to OPLL. Positive evidence of linkage were found at limited number of loci and the best evidence of linkage was detected at D21S263 on chromosome 21q21.3-22 (maximum LOD score 3.6). In order to identify the susceptible gene for OPLL, we performed extensive association studies with SNPs (single nucleotide polymorphisms) covering the 15 Mb of linkage region. SNPs were obtained from the public database (NCBI dbSNP [<http://www.ncbi.nlm.nih.gov/SNP/>] and IMS-JST JSNP DATABASE [<http://snp.ims.u-tokyo.ac.jp/>]). We have analyzed 189 genes with 400 SNPs in the region. Thus far we have obtained positive allelic associations with 6 genes ($P < 0.05$). Linkage disequilibrium and haplotype analysis could infer that one gene could be responsible for the predisposition of OPLL. Identification of the susceptibility of OPLL by genome-wide linkage study and allelic association study could provide new insights into the pathogenesis of OPLL, which could lead to development of novel therapeutic tools.

BDNF and susceptibility to autism spectrum disorders. *S. Robitaille*¹, *X. Liu*^{1,2,3}, *M. Currie*³, *J.J.A. Holden*^{1,2,3}. 1) Department of Physiology, Queen's University, Kingston, ON, Canada; 2) Department of Psychiatry, Queen's University, Kingston, ON, Canada; 3) Cytogenetics and DNA Research Laboratory, Ongwanada, Kingston, ON, Canada.

Autism Spectrum Disorders (ASDs) are a group of neurodevelopmental disorders characterized by impairments in reciprocal social interaction and communication as well as repetitive behaviours. Nelson and colleagues¹ demonstrated significant elevation of the brain-derived neurotrophic factor (BDNF) neuropeptide in neonatal blood from individuals later diagnosed with autism and/or developmental disabilities. These findings suggest that BDNF is a good candidate gene for ASDs. Philippe and colleagues² recently reported their findings on an intragenic marker and two markers linked to BDNF in 39 multiplex autistic families, finding no evidence for an association between BDNF and autism. We investigated the same intragenic marker and a closely linked marker, D11S1301, in 123 multiplex ASD families and a comparison group (N=171). Although the allele distributions for both markers did not differ in ASD families compared to controls, we found that the D11S1301-331 allele and the BDNF-D11S1301 170-331 haplotype were transmitted more often than expected to offspring using the family-based association test (FBAT) and the Transmit program (331 allele: S=141, E(S)=122.5, p=0.013; 170-331 haplotype: $c^2=11.359$, df=1, p=0.001). Moreover, fathers transmitted the 331 allele to offspring more often than the 50% expectancy ($c^2=3.928$, df=1, p=0.047). Our findings support BDNF or a closely linked unidentified gene as a candidate gene for autism. Genotyping of another intragenic BDNF marker with an amino acid change (ie. valine-66-methionine) is in progress. (Supported by a scholarship to SR from NSERC and research grants to JJAH from CIHR and OMHF) ¹ 2001 Ann Neurol 49: 597-606. ² 2002 Am J Med Genetics 114: 125-128.

Optineurin Sequence Variants Do Not Predispose to Primary Open Angle Glaucoma. *J.W. Walter¹, R.R. Allingham¹, J.D. Flor¹, K.R. LaRocque¹, F.L. Graham¹, B. Broomer¹, E.A. del Bono², J.L. Haines³, M.A. Pericak-Vance¹, M.A. Hauser¹, J.L. Wiggs².* 1) Duke Univ Medical Ctr, Durham, NC; 2) Massachusetts Eye and Ear Infirmary, Boston, MA; 3) Vanderbilt University, Nashville, TN.

The optineurin protein, which is encoded by the OPTN gene located on chromosome 10p14-p15, is expressed in the brain and many tissues of the eye including the trabecular meshwork, retina, and non-pigmented epithelium. Recent studies have suggested that mutations and polymorphisms in optineurin may cause or increase susceptibility to normotensive glaucoma (Rezaie T et al., Science 295, 1077). To assess the influence of optineurin in the more common high-tension primary open angle glaucoma (POAG), we screened the probands from a set of 86 adult-onset POAG families. Criteria for inclusion include age of onset great than 35 years, elevated interocular pressure ³ 22mm Hg in both eyes, optic nerve damage, and corresponding visual field loss in at least one eye. Transgenomic WAVE denaturing high performance liquid chromatography (dHPLC) was used to screen for sequence variants in the optineurin gene. Pools of genomic DNA from 3 individuals were subjected to dHPLC at several different temperatures. Pools displaying altered column retention time were sequenced to confirm and identify sequence variants. Exons 4 and 5, containing the previously reported E50K and M98K variants, were sequenced in their entirety. Within this sample of 86 probands and 80 controls, we detected the M98K variant in 10% of affected individuals, as well as 10% of controls. We did not detect the E50K variant in any of the individuals tested nor did we detect any other polymorphisms within the optineurin coding sequence. However, we did detect six intronic SNP's none of which are predicted to cause splice defects. This data suggests that variations in the optineurin gene do not cause or predispose individuals to adult onset POAG.

Characterization and Analyses of AD Candidate Genes P16 and MTAP on Chromosome 9p21. *P.-T Xu¹, J.R. Gilbert¹, S.N. Walters¹, C. Browning¹, K.A. Desombre¹, K. Nicodemus¹, W.K. Scott¹, J.L. Haines², M.A. Pericak-Vance¹.* 1) Duke University Medical Center, Durham, NC; 2) Vanderbilt University Medical Center, Nashville, TN.

The Collaborative Alzheimer Project (CAP) has completed an Alzheimers disease (AD) screen using 334 microsatellite markers in the largest AD dataset to date (455 families, 726 sibpairs). A region on chromosome 9p21, using the marker D9S741, gave the highest lod score (MLOD = 3.43; MLS = 3.31 in the overall dataset and MLOD = 3.94; MLS = 4.41 in autopsy confirmed cases). We have found evidence for significant association with SNPs around the CDKNA2 (p16) [two point results (rs1042387 (p=0.0006) and rs 717326 (p=0.04)] and Methylthioadenosine Phosphorylase (MTAP) genes [multipoint results:tsc03520892/tsc0129737_1/tsc0129737_2 (p=0.003)].

The region from the p16/p15 complex to MTAP encompasses approximately 200 kb. P16, which co-localizes with nNOS and p21^{ras} in pyramidal neurons in AD brain is an approximately 7kb tumor suppressor gene that produces a 987 bp transcript and has three exons. It utilizes a different promoter but shares portions of its exon 1, exon 2, and part of exon 3 with an overlapping p14 isoform/gene.

We have analyzed the AD candidate genes P16 and MTAP by sequencing the whole p16 gene, and the coding region of MTAP gene including exons and exon/intron junctions. We have designed 34 pairs of primers that covered a region of 15,525 bp of p16 gene. We have found 6 new SNPs in several AD patients in which 2 SNPs are located about 100 bp and 4,000 bp upstream of exon 1, respectively. We have also identified 13 isolated nucleic variants in P16 gene. A poly-(T) tract with a variation from 15T to 53T in different AD patients was also detected in intron1 of P16 gene. A dinucleotide (CA) repeat was observed in intron1b ~200 bp downstream of exon1b of p14 protein gene (an isoform of p16). We have found 4 new SNPs in exon 3 and exon 8 of MTAP gene including a missense mutation (valine to isoleucine) in coding region of exon3.

The role of promoter polymorphisms in the APOE gene in progression of multiple sclerosis (MS). *S. Schmidt¹, I.C. Allen¹, R.R. Lincoln², J.B. Rimmler¹, L.F. Barcellos², A. Swerdlin², A.M. Saunders¹, J.R. Oksenberg², S.L. Hauser², J.L. Haines³, M.A. Pericak-Vance¹.* 1) Duke Univ Med Ctr, Durham, NC; 2) Univ California, San Francisco, CA; 3) Vanderbilt Univ Med Ctr, Nashville, TN.

Genes on chromosome 19q13 have been implicated in both progression and risk of MS. For risk, linkage evidence implicates a broad region, and association evidence implicates genes distal to the apolipoprotein E (APOE) locus. For progression, we have previously reported evidence that the APOE-4 allele is associated with a more severe disease course, whereas carriers of the APOE-2 allele are more likely to be affected with a mild form of MS. In addition to the functional polymorphism that produces the well-known apoE protein isoforms, we have now completed genotyping of three promoter, one intronic, and one 3' UTR polymorphism in the APOE gene (-491A/T, -427C/T, -219G/T, 113C/G, 5361C/T) in a large data set of Caucasian MS families. The 219T allele has previously been reported to increase risk of both Alzheimer disease and myocardial infarction independently of the established effect of the APOE-4 allele in these disorders. Both -219G/T and -491A/T have been shown to influence the transcriptional activity of the APOE gene. Our current MS data set consists of 189 multiplex and 410 singleton families, with a total of 872 clinically definite MS patients and 1404 unaffected family members. Preliminary analyses of the additional APOE variants indicate that none of them are associated with an increased risk of MS, and that they are not independently associated with disease course. Haplotype analyses suggest that the -219T and -491A allele in combination with APOE-4 are significantly over-represented in patients with a more severe course of MS (EDSS>6.0 within 10 years of disease onset; $p=0.01$, $p=0.01$, resp.). On the other hand, -491A in combination with APOE-2 is significantly more frequent in patients with mild MS (EDSS<3.0 after 10 or more years of disease duration; $p=0.04$). While the strength of the association is similar to that observed with APOE-4 and APOE-2 alone, the particular associated alleles of the promoter variants further support a role of the APOE gene in MS progression.

Germline mutations in angiotensinogen associated with predisposition to type 1 diabetes. *D.L. Russell¹, F. Kronenberg², K.M. Timms¹, M.T. McDermott¹, J. DeGrado¹, H.A. O'Neill¹, Y. Chen¹, M. Auinger³, C. Säly⁴, R. Sommer⁵, R. Weitgasser⁶, S.C. Hunt⁷, M. Skolnick¹, M. McGrail¹, D.M. Shattuck¹.* 1) Myriad Genetics, Salt Lake City, UT; 2) Institute of Med Biol and Human Genetics, Univ of Innsbruck, Austria; 3) Lainz Hospital, Vienna, Austria; 4) Feldkirch Hospital, Dept of Inter Med, Austria; 5) Graz Univ Hospital, Dept of Inter Med, Austria; 6) Salzburg Hospital, Dept of Inter Med, Austria; 7) Cardiovascular Genetics, Univ of Utah, Salt Lake City, UT.

Type 1 diabetes (T1D) is an autoimmune disease with both genetic and environmental contributing factors. Reportedly, >20 loci show linkage to T1D. The HLA complex (IDDM1) has the most significant association but accounts for ~50% of the familial clustering of T1D, indicating the existence of non-HLA, T1D-predisposition genes. There is significant evidence of linkage to T1D at 1q42-43, near angiotensinogen (*AGT*). To investigate whether *AGT* is the gene responsible for this linkage, we obtained 38 nuclear families, each with > 2 children affected with T1D, from the HBDI. We observed haplotype sharing across 1q42-43 in 20 families, which we used to screen for mutations in *AGT*. We detected rare novel mutations, including Cys18Arg. Next, we screened these variants in an independent set of 308 unrelated cases and 1003 population controls. Enrichment of Cys18Arg in the cases as compared to controls (3/308 vs. 1/1003, $p=0.03$) was detected. We then sequenced the promoter and coding region of *AGT* in the cases and 631 of the controls. In cases, we found novel rare missense and non-coding mutations, including three variants in a putative HNF4/MODY1 activating element. Comparison of the frequency of rare mutations in *AGT*, in both sets, showed significantly more carriers in the cases as compared to controls (18/308 vs. 17/631, $p=0.01$). These data support our hypothesis that the mutations, described here, in *AGT* are predisposing to T1D. To replicate these results in an independent study population, we are screening for Cys18Arg and the HNF4 activating element variants in a set of Austrian type 1 diabetics and a set of matched controls of the same ethnicity and from the same geographical region.

Linkage disequilibrium mapping of the serotonin transporter gene (SLC6A4) region in autism. *J.E. Vander Molen¹, S.J. Kim², E.H. Cook², N. Cox¹*. 1) Human Genetics, University of Chicago, Chicago, IL; 2) Laboratory of Developmental Neuroscience, Child and Adolescent Psychiatry, Department of Psychiatry, University of Chicago, Chicago, IL.

The serotonin transporter gene (SLC6A4) is a candidate gene in autistic disorder based on neurochemical studies and the efficacy of serotonin transporter inhibiting drugs. Previous studies have shown nominally significant over/under-transmission of alleles at this locus [Kim et al, Mol Psych, 2002]. A sample of 115 trios consisting of unaffected parents and an offspring affected with autism were typed for 27 markers in and around the SLC6A4 region, spanning 2.1 Mb. We applied the decay of haplotype sharing (DHS) method developed by McPeck and Strahs [AJHG, 1999] to these data to localize variation affecting susceptibility to autism and identify ancestral haplotypes. The 95% confidence interval obtained using the conditional coalescent model for the DHS analysis is a 0.51 Mb region including markers D17S1294 through 5-HTTLPR. The ancestral haplotype for markers in the 95% CI is largely consistent with results of the TDT for these data using individual markers, including the allele found overtransmitted for all markers except HTT SNP17 in exon 1b and a GAA repeat in intron 7. DHS analyses with control data only show a low peak for the likelihood function when compared to analyses with patient and control data, indicating that DHS adequately models background LD in this region. Pairwise LD between markers on patient chromosomes appears to be of greater magnitude than LD on the untransmitted parental chromosomes.

CARD4/NOD1 in Inflammatory Bowel Disease. *H. Zouali¹, S. Lesage¹, F. Merlin¹, JP. Cézard², JF. Colombel³, J. Belaiche⁴, S. Almer⁵, C. Tysk⁶, C. O'Morain⁷, M. Gassull⁸, R. Modigliani⁹, C. Gower-Rousseau³, M. Chamaillard¹, G. Thomas¹, JP. Hugot¹.* 1) Fondation Jean Dausset, Paris, France; 2) Hôpital Robert Debré, Paris, France; 3) Hôpital Calmette, Lille, France; 4) Department of Gastroenterology, CHU de Liège, Belgium; 5) Institutionen för Molekylär och Klinisk Medicin, Linköpings Universitet, Linköping, Sweden; 6) Department of Gastroenterology, Örebro Medical Center Hospital, Örebro, Sweden; 7) Department of Gastroenterology, Adelaide & Meath Hospital, Dublin.; 8) Department of Gastroenterology, Hospital Universitari Germans Trias i Pujol, Badalona, Spain; 9) Department of Gastroenterology, Hôpital Saint Louis, Paris, France.

Background : IBD are complex genetic disorders of unknown etiology. We have recently identified CARD15/NOD2 as being a susceptibility gene for Crohns disease (CD). Aim : Because CARD4/NOD1 shares many structural and functional similarities with CARD15/NOD2, we tested its putative role in Inflammatory Bowel Disease (IBD). Patients and methods : The IBD families were recruited through a large European consortium. The 11 exons and intron-exon boundaries of CARD4/NOD1 were screened for the presence of variants in 77 unrelated IBD patients (62 CD and 15 UC patients) using direct sequencing. The identified variant was genotyped using a PCR-RFLP procedure. The Transmission Disequilibrium Test (TDT) was computed by GENEHUNTER 2.0 program. Results : Nine sequence variations were identified in the coding sequence of the CARD4 gene. Five of them (E266K, D372N, R705Q, T787M and T787K) were non conservative variants but only one (E266K) was present in more than one IBD patient. This variant was genotyped in 291 IBD families including 235 Crohn (CD) and 56 Ulcerative Colitis (UC). TDT failed to demonstrate any association between the E266K variant and any of the three phenotypes : IBD, CD and UC ($p>0.05$). The analysis of the phenotype-genotype relationship do not show any differences between mutated E266K and wild type IBD patients. Conclusion: These results suggest that CARD4/NOD1 do not play a major role, if any, in IBD genetic susceptibility.

A Tnfr1 genotype with a protective role in familial rheumatoid arthritis. P. Dieude¹, E. Petit-Teixeira¹, S. Moreno¹, J. Osorio¹, S. Cailleau-Moindrault¹, C. Stalens¹, S. Lasbleiz², B. Prum³, T. Bardin^{1,2}, F. Cornelis^{1,2}. 1) Laboratoire ECRAF-GENHOTEL, Universites ParisVII-Evry, Evry, France; 2) Unite de Genetique Clinique, Hopital Lariboisiere, Assistance Publique des Hopitaux de Paris, France; 3) Laboratoire Statistique et Genome, Genopole, Evry, France.

Background Tumor necrosis factor alpha is involved in rheumatoid arthritis (RA) and binds TNFR1 and TNFR2 receptors. Genome scans have suggested the TNFR1 and TNFR2 loci as susceptibility loci for RA. Previous studies in a UK and French Caucasian population have shown an association between a TNFR2 genotype and familial, but not sporadic RA. Our objective was to test the TNFR1+36 A/G polymorphism for an association in RA.

Methods. To test for an association in sporadic RA, 100 families with one RA patient and both parents were genotyped for the +36 A/G polymorphism. Analysis used the transmission disequilibrium test and the haplotype relative risk (HRR). To test for an association in familial RA, RA index cases from affected sib pair (ASP) families that had been typed for two TNFR1 flanking microsatellite markers (D12S99 and D12S1625) (n = 86) were genotyped for +36 A/G polymorphism. To further explore the involvement of a particular genotype in RA ASP families, we used a subgroup more likely to have a TNFR1 putative factor : RA index cases with TNFR1 twin like RA sib (n = 19) : a TNFR1 genetically identical RA affected sib (RA cases for whom the affected sib had identical genotype at both TNFR1 flanking microsatellites).

Results. A trend of decreased number of +36 A/A genotype was observed in sporadic RA (33%) compared to controls (41%) and reached significance in familial RA (24%) (P= 0.012). This negative association was most marked in the context of TNFR1 twin-like RA sibs, 11% compared to 41% in the controls (P= 0.008).

Conclusion. This study provides evidence of the involvement of TNFR1 in RA genetic heterogeneity. Our data suggest that a TNFR1 recessive factor, in linkage disequilibrium with the +36A allele, plays a protective role in a subset of families with multiple RA cases.

Multivariate analysis of autoantibody repertoires in Systemic Lupus Erythematosus (SLE) multiplex families. C. Fesel¹, M. Barreto¹, F. Fontes¹, R. Andreia², F. Crespo², C. Vasconcelos², AM. Vicente¹, C. Ferreira². 1) Instituto Gulbenkian de Ciencia, Oeiras, Portugal; 2) Associao de Doentes com Lupus, Lisboa, Portugal.

SLE is an autoimmune disease with female predominance and demonstrated inheritable effects, though complex and most likely highly multigenic, and not yet well elucidated by genetic studies. Patients produce IgG autoantibodies to a wide variety of primarily nuclear, but also non-nuclear autoantigens, likely to include yet uncharacterized ones. In order to identify genetic factors influencing SLE, we have applied an indirect strategy to first dissect serological subtypes without respect to known antigens, expected to represent non-genetic as well as genetic effects, but with the possibility to find among them sub-phenotypes likely to be major Mendelian, thus useful for mapping. Using a standardized quantitative immunoblot method, we measured serum IgG autoantibody repertoires to 3 tissue extracts, permitting to quantify 130 separate reactivity bands in a sample of 16 multiplex families including 27 patients and 71 unaffected relatives, and 46 healthy control adults. Principal component analysis on these parameters showed that among the 10 major principal components extracted, 4 were associated with lupus, one of which was interpretable as the representation of a genetic trait. This component (factor 10), though among the 4 lupus-associated factors contributing least to the total data variance (3%), was like the other three strongly associated with lupus ($p=.003$, Mann-Whitney test), but, unlike them, had a heritability estimate of 50% ($p=.003$). Family correlations (mother-daughter .41, father-daughter .58, while parent-son and parent-parent insignificant) were most compatible with the interpretation as a trait with sex-dependent penetrance. This type of model was also the best-fitting, and non-rejectable Mendelian model for factor 10 in complex segregation analysis. Though requiring confirmation in a larger sample, this result supports the existence of subtypes characterized by autoantibody reactivity patterns, and their possible usefulness to constitute a phenotypic trait appropriate for mapping.

Paternal origin of Y-chromosome-bearing cells in microchimeric nulligravid females with autoimmune disease.
C.J. Osgood^{1,2}, L. Blanchfield¹, R.N. Slotnick³, M.W. Stacey², C. Gabriel^{2,4}, D.S. Lidwell². 1) Dept Biological Sci, Old Dominion Univ, Norfolk, VA; 2) Dept of Pediatrics, Eastern Virginia Medical School, Norfolk, VA; 3) Dept OB/GYN, Eastern Virginia Medical School; 4) Dept Internal Medicine, Eastern Virginia Medical School.

Autoimmune diseases (AID) are estimated to affect approximately 30 million people in the US, with a predominance of affected females. The etiology of autoimmune diseases is poorly understood, although several authors have noted an association between microchimerism and enhanced risk of developing disease. We have detected evidence of presumptive microchimerism in samples collected from nulligravid, juvenile females diagnosed with AID. We have detected Y-chromosome sequences in samples from patients diagnosed with juvenile rheumatoid arthritis, dermatomyositis and system lupus erythromatosis. We report here the results from DNA sequence analyses of Y-chromosome sequences detected in those females by PCR. We find that 1) the PCR-amplified sequences are not attributable to contamination, and 2) that those sequences match uniquely to the fathers of the nulligravid females. We propose that nulligravid females are likely derived from XXY zygotes with subsequent loss of the Y-chromosome in the majority cell population. Possible mechanisms by which such events promote development of AID will be discussed.

Association of a functional repeat polymorphism within the promoter of the human Nramp1 gene in familial rheumatoid arthritis. E. Petit-Teixeira¹, P. Dieude¹, V. Arbelaziz¹, J. Osorio¹, C. Pierlot¹, S. Cailleau-Moindrault¹, S. Lasbleiz², T. Bardin^{1,2}, F. Cornelis^{1,2}. 1) Laboratoire ECRAF-GENHOTEL, Universités Paris VII - Evry, Evry, France; 2) Unité de Génétique Clinique, Hôpital Lariboisière, Assistance Publique des Hôpitaux de Paris, France.

The gene Nramp1 (Natural resistance associated macrophage protein 1) encodes a protein which regulates macrophage activation in infectious and auto-immune diseases. Our objective is to test one promoter polymorphism of this gene in rheumatoid arthritis (RA).

Methods To test for an association of this functional polymorphism with sporadic RA, 100 families with one RA patient and both parents were genotyped. Analysis used the transmission disequilibrium test (TDT) and the haplotype relative risk (HRR). To test for an association in familial RA, RA index cases from 84 affected sibling pairs (ASP) families were also genotyped. To further explore the involvement of a particular genotype in RA ASP families, we used a subgroup (n = 20) more likely to have a NRAMP1 putative factor : RA index cases with NRAMP1 "twin like" RA sibling (RA cases for whom the affected sibling had identical genotype at both NRAMP1 flanking microsatellites).

Results No allelic association for the three alleles detected was observed with sporadic RA. The HRR test in sporadic RA found a trend for increase of the 2/2 genotype present 6 times in patients whereas never in the controls ($P = 0.051$). In familial RA, the suspected genotype was significantly more frequent than in controls (14% vs 0%, $P = 5.10^{-5}$). In ASP index cases with NRAMP1 "twin like" RA sibling, this genotype was also more frequent than in controls (10% vs 0%, $P = 0.026$).

Conclusion The increased frequency of the 2/2 genotype observed in familial RA cases provided evidence for a factor involved in RA genetic heterogeneity. Our data suggested that a NRAMP1 recessive factor, in linkage disequilibrium with the allele 2, would play a role in RA genetic susceptibility.

DNA methylation imprints of the maternally expressed genes differs from the paternally expressed genes in the AS/PWS region. *H.J. Dong, J.R. Khadake, D. Amidon, M.M. Whitney, D.J. Driscoll.* Pediatric Genetics, Univ. of Florida, Gainesville, FL.

Angelman syndrome (AS) and Prader-Willi syndrome (PWS) are clinically distinct imprinted gene disorders that map to the chromosome 15q11-q13 region. PWS results from paternal insufficiency while AS results from maternal insufficiency. Paternally expressed genes in the CpG islands of the "PWS region" are undermethylated in comparison to the maternally repressed alleles. Since the maternally expressed *UBE3A* and *ATP10C* genes in the "AS region" both have prominent CpG islands we looked for parent-of-origin methylation differences in various AS and PWS tissues and normal germ cells. Previous *UBE3A* analysis by our group using Southern blot hybridizations had not revealed any DNA methylation in somatic tissues and germ cells. In order to do an in-depth analysis of the 144 individual CpG dinucleotides in the 1140 bp CpG island of *UBE3A* we performed genomic sequencing by the sodium bisulfite method in fibroblast and several brain regions from AS (paternal-only allele) and PWS (maternal-only allele) deletion individuals. The *ATP10C* CpG island was analyzed in various somatic tissues, including brain, as well as in adult male germ cells and fetal ovaries using Southern blot analysis by CpG island probes.

We found that the entire CpG island for *UBE3A* is unmethylated by genomic sequencing in fibroblast and brain tissues for both the maternal and paternal alleles. For *ATP10C* there is variable DNA methylation in the CpG island of various somatic tissues and the brain, but it is not parent-of-origin specific. However, adult male germ cells are methylated in the CpG island while the fetal oocytes are unmethylated. Thus, in contrast to the paternally expressed genes in the AS/PWS region, we did not find any DNA methylation imprints in somatic tissues from the maternally expressed genes, *UBE3A* and *ATP10C*. However gametic imprints were found in *ATP10C*, but not *UBE3A*. Therefore, the imprinted expression of the genes in the "AS region" are probably regulated in somatic tissues by different mechanisms than those in the "PWS region".

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Strain-dependent differences in phenotype in the Prader-Willi syndrome mouse model. *S.J. Chamberlain, C.I. Brannan.* Molecular Genetics & Microbiology and the Center for Mammalian Genetics, University Florida, Gainesville, FL.

Prader-Willi syndrome (PWS) is a contiguous gene syndrome resulting from deletion or mutation of two or more imprinted genes. Previously, we described a mouse model for PWS in which paternal transmission of a 35 kb PWS Imprinting Center (PWS-IC) deletion mutation resulted in complete loss of local paternal gene expression. On the C57BL/6J genetic background, these PWS mice exhibit severe growth retardation, hypotonia, and neonatal lethality. We later found that (FVB/NJ x C57BL/6J) F1 mice harboring the PWS-IC deletion mutation are able to survive to adulthood, provided the majority of the wild-type sibs are removed within a few days after birth. Low-level expression of various genes in the PWS region that was detectable by RT-PCR was seen in the IC deletion offspring from an FVB/NJ mother, suggesting that low-level gene expression from the PWS region was allowing PWS pups to survive.

We sought to determine if either survival or low-level leaky gene expression was associated with other mouse strains, some of which were known to be non-methylators of transgenes. To this end, we tested C3H/HeJ, Balb/c, 129/Sv, and DBA/2J mice for similar leaky gene expression in the PWS region, as well as survival of PWS mice on these strain backgrounds.

Microarray analysis of gene/transcript expression in Prader-Willi syndrome: Deletion versus UPD. *D. Bittel, N. Kibiryeve, Z. Talebizadeh, M.G. Butler.* Children's Mercy Hospitals and Clinics and University of Missouri-Kansas City School of Medicine, Kansas City, MO.

Prader-Willi syndrome (PWS) is characterized by infantile hypotonia, hypogonadism, hyperphagia, early onset of childhood obesity and mental deficiency. PWS is caused by a deficiency of expression of genes located on the proximal long arm of chromosome 15. Several genes have been identified in the 15q11-q13 region which are imprinted and the lack of the paternally expressed genes contributes to PWS. A deletion of 15q11-q13 is found in about 70% of PWS subjects, maternal uniparental disomy (UPD) 15 in approximately 25% and an imprinting mutation in 2-5%. In our continuing efforts to understand the molecular genetics of PWS we have generated cDNA microarrays representing most of the genes/transcripts from the PWS critical region. We have used our custom microarrays to compare gene expression from actively growing lymphoblastoid cell lines established from nine adult males [6 with PWS (3 with deletion and 3 with UPD) and 3 controls]. There was no evidence of expression of genes previously identified as paternally expressed (e.g., SNRPN, IPW, MAGEL2, ZNF127 and NDN) in the PWS cell lines and we detected no difference in expression levels of genes with biallelic expression located outside of the 15q11-q13 region (e.g., B-ACTIN, FIBRILLIN and GAPDH) in all cell lines studied. There was no difference in expression levels of biallelically expressed genes (e.g., OCA2) from within the 15q11-q13 when comparing UPD cell lines with controls. Two genes previously identified as maternally expressed (UBE3A and ATP10C) showed an approximate 2 fold increase in expression in UPD cell lines compared with controls and PWS deletions. In addition, several transcripts (e.g., SGC32610, WI-18493 and D38449) had increased expression in UPD cell lines compared to controls, indicating maternal bias. These transcripts may also contribute to physical and behavioral differences recently observed between PWS deletion and UPD patients and/or in Angelman syndrome, an entirely different clinical disorder and also due to genomic imprinting of this chromosomal region.

Use of a marked transgene to study imprinting in the Prader-Willi syndrome region. *C. Futtner, S.J. Chamberlain, M. Elmore, C.I. Brannan.* Molecular Genetics & Microbiology and the Center for Mammalian Genetics, University of Florida, Gainesville, FL.

The Prader-Willi syndrome (PWS) candidate genes as well as the Angelman syndrome (AS) candidate gene are regulated by an Imprinting Center (IC) located 5' and including the first exon of the SNRPN gene. The elements that are necessary for the IC have been defined by means of deletion mutations that have been mapped in humans and created in mice. We then determined the minimal sequence sufficient to confer imprinting to a transgene. A single-copy transgene containing 90 kb of upstream sequence are expressed upon paternal, but not maternal transmission, while transgenes containing 8kb and 23 kb of upstream sequence do not demonstrate this imprinted expression pattern. These data suggest that the relevant sequences for single copy imprinting lie within a 90 kb region upstream of Snrpn.

To further investigate elements that are minimally sufficient to confer correct imprinted expression in single-copy transgenes, we have taken advantage of a BAC modification system based on the Red genes from lambda phage (1). We subjected a BAC, containing the necessary upstream sequences for imprinting, to two different modifications. First of all, the murine Snrpn exon 1 was replaced with human SNRPN exon 1. The purpose of this modification is to determine whether or not the murine imprinting machinery was capable of acting on human sequences. Secondly, the exons 5-7 of Snrpn were replaced with a neomycin resistance cassette. This modification was performed to allow for the analysis of transgene expression upon both paternal and maternal inheritance in a single generation.

1. Lee, E.C., et al. (2001) *Genomics* 73:56-65.

Epimutation at KvDMR1 leads to ablation of *CDKN1C* expression in patients with Beckwith-Wiedemann syndrome. *N. Diaz-Meyer*¹, *C.D. Day*¹, *E.R. Maher*², *C. Junien*³, *G. Graham*⁴, *V. der Kaloustian*⁵, *M.J. Higgins*¹. 1) Roswell Park Cancer Institute, Buffalo, NY; 2) University of Birmingham, Birmingham, UK; 3) Inserm UR383, Hôpital Necker Enfants Malades, Paris, France; 4) Children's Hospital of Eastern Ontario, Ottawa, ON, Canada; 5) Montreal Children's Hospital, Montreal, PQ, Canada.

Imprinted genes are expressed from only one allele depending on whether it was inherited from the mother or father. An imbalance in the expression of imprinted genes in human chromosome 11p15.5 results in Beckwith-Wiedemann syndrome (BWS), an overgrowth and cancer predisposition condition. Together, uniparental disomy (UPD), duplications and translocations of 11p15.5, inactivating mutations in *CDKN1C* (*p57^{KIP2}*), and loss of imprinting of *IGF2* account for fewer than half the cases of BWS. We and others have shown that the majority of remaining cases are associated with demethylation of KvDMR1, a differentially methylated CpG island in 11p15.5. This locus has several characteristics of an imprinting control region (ICR) including the derepression in *cis* of normally silent (imprinted) genes following its deletion on the paternal chromosome, as well as methylation-modulated chromatin insulator activity in cell culture. Thus, it appears that the function of the unmethylated, paternally-inherited KvDMR1 is to silence genes under its control on the paternal chromosome, thereby giving rise to maternal-specific imprinted expression. Although direct evidence is lacking, it has been proposed that demethylation of KvDMR1 on the maternal chromosome in patients with BWS results in aberrant activation of this repressive function which leads to the pathological silencing of maternally expressed genes in 11p15.5. To test this hypothesis, we are performing real-time quantitative RT-PCR using TaqMan assays. As predicted by the model, early results show that *CDKN1C* is significantly downregulated in fibroblast cells from BWS patients that lose methylation at KvDMR1. This epigenetic repression is functionally equivalent to an inactivating mutation in *CDKN1C* and would likely lead to the syndrome. Funded by NCI/NIH Ca63333 to MJH.

***Atp10a*, the mouse homologue of the human imprinted gene, *ATP10C*, escapes genomic imprinting. T.**

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Mouse *Atp10a* (GenBank accession No. AF156549), previously called *pfatp* gene encodes a member of the third subfamily of P-type ATPases, characterized by a putative aminophospholipid transporter. *Atp10a* is located between *Gabrb3* and *Ube3a/Ipw* on mouse chromosome 7 and is a candidate gene for increased body fat seen in mice only with deletions of the maternally derived *p*-locus. Such positional candidacy neighboring the maternally expressed gene *Ube3a* and an unusual inheritance pattern of the obese phenotype with *p*-locus deletions have suggested that *Atp10a* might be imprinted. Recently, its human homologue, *ATP10C*, was identified as a second imprinted, maternally expressed gene, 200 kb distal to *Ube3a* at 15q11-q13. Although preferential maternal expression of *ATP10C* has been demonstrated in fibroblasts and the brain, no direct evidence of *Atp10a* imprinting has been reported yet. To know the imprinting status of *Atp10a*, we performed expression analysis in various tissues from embryo and adult mice of reciprocal crosses between C57BL/6 and PWK (a divergent strain of *Mus musculus*). RT-PCR using intron-spanning primer pairs in exons 10 and 11, followed by *Hpy*CH4IV digestion, revealed that *Atp10a* was ubiquitously and biallelically expressed in all the embryonic and adult tissues examined, including various brain regions of adult mice and white adipose tissues. Although we can not completely exclude the possibility of mouse-strain-specific imprinting or brain-region-specific partial imprinting, our results suggest that mouse *Atp10a* escapes genomic imprinting, and that other maternally expressed genes in the *p*-locus deletion interval may cause the obesity phenotype.

Identification of potentially imprinted genes on human chromosome 14. *G. Macintyre, C.J. Clarke, P. Callum, D.W. Cox.* Department of Medical Genetics, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

Genomic imprinting is the differential expression of the two alleles of a gene, predominantly from either the maternally or the paternally inherited allele. In mammals, imprinting affects many fundamental processes involved in development, growth and nurturing behaviour. Patients exhibiting paternal or maternal uniparental disomy for chromosome 14 (pat or mat UPD14) display distinct disease phenotypes, suggesting the presence of imprinted genes on chromosome 14. Reciprocally imprinted genes, *DLK1* and *GTL2*, have been identified at 14q32. It has been suggested that *DLK1* and *GTL2* constitute part of a cluster of imprinted genes. How far this imprinted region extends is unknown. Interestingly, two previously reported cases of interstitial segment matUPD14, proximal to and not including 14q32, describe a phenotype that is similar to matUPD14, suggesting that more proximal regions of chromosome 14 may also contain differentially expressed genes. We have used RNA from cell lines derived from UPD14 patients, human chromosome 14 monochromosomal hybrids and human fibroblasts in an RT-PCR assay to identify candidate genes for genomic imprinting. Our analysis has focused on the expression of genes and ESTs situated within 14q12-13, 14q22-24 and 14q32.11-32.33. The latter region is adjacent and proximal to the known imprinted region. More than 250 genes and ESTs have been analysed giving extensive coverage (approximately 50-400kb) throughout these regions. No evidence for imprinting was found in the region adjacent to the imprinted cluster. However, a number of genes and ESTs in the more proximal regions of chromosome 14 exhibit differential gene expression in some of the cell lines tested and are potentially imprinted genes. How the phenomenon of differential gene expression relates to the function of the protein products of these genes will be discussed.

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Species specificity of the Prader-Willi syndrome imprinting center. *K.A. Johnstone, M. Elmore, C.I. Brannan.*
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Genomic imprinting is the differential expression of genes dependent on parent of origin. The majority of imprinted genes identified to date are conserved between mouse and human. Most imprinted genes reside in clusters and this appears to be key to the imprinting process. While the precise mechanism of imprinting is likely to differ between clusters, the mechanism active within a cluster is superficially conserved between species. The genes in the Prader-Willi (PWS) and Angelman syndrome (AS) region on chromosome 15q11-q13 are coordinately regulated by two imprinting centers (ICs), the PWS-IC and the AS-IC. We have previously shown using a mouse model that the PWS-IC is functionally conserved. Our recent data shows that a single-copy murine transgene is correctly imprinted and therefore defines the region likely to contain the murine AS-IC. We have previously shown that a human transgene fails to imprint in mice regardless of copy number and despite containing sequences from both the AS-SRO and the PWS-SRO. This has led us to propose that, although there is a high degree of conservation of imprinting within the region, the imprinting machinery has diverged.

In order to determine which processes have diverged, we have replaced *Snrpn* exon 1 and adjacent sequences with an equivalent human region spanning the PWS-SRO by gene targeting in ES cells. The resulting mouse model should allow us to study in more detail the sequences and factors involved in the acquisition and maintenance of imprinting in the PWS and AS region.

DNA methylation patterns in the *Snurf-Snrpn* gene 5' CpG island during embryonic and germ cell development.

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Differential DNA methylation plays a critical role in the epigenetic mark (imprint) that establishes and maintains allele-specific imprinted gene expression. We have analyzed the DNA methylation patterns in the 5' CpG island of the imprinted mouse *Snurf-Snrpn* gene, a paternally expressed gene in the Prader-Willi and Angelman Syndromes (PWS/AS) region of mouse chromosome 7c. This analysis utilized sodium bisulfite genomic sequencing to examine 41 CpGs within the entire length of the 1.1 kb CpG island using species-specific polymorphisms from inter-species hybrids to distinguish the parental alleles. In adult brain and spleen, the maternal allele is methylated and the paternal allele is unmethylated at all CpGs analyzed. During germ cell development, methylation imprints must be erased and reset to ensure that the zygote receives one allele of each parental epigenotype. Analysis of the same region in primordial germ cells (PGCs) from E13.5 indicates that the maternal DNA methylation imprint is completely erased (ie. unmethylated) in both male and female PGCs by this stage of germ cell development. Analysis in pachytene spermatocytes and round spermatids shows that both alleles remain unmethylated through these stages of spermatogenesis. Thus, erasure occurs before germ cells reach the genital ridge and resetting of the imprint occurs at a later stage. During embryogenesis, imprinted alleles must also maintain their parent-specific epigenotype during the global demethylation that occurs in the pre-implantation embryo. Our analysis of blastocysts (near the end of global demethylation) indicates that the entire adult somatic methylation imprint of the *Snurf-Snrpn* CpG island is retained and remains through E9.5 and E12.5 after implantation and remethylation of the genome. Thus, the overall CpG methylation pattern in the *Snurf-Snrpn* 5' CpG island escapes global demethylation and is likely to contribute to the maintenance of allele-specific imprinted gene expression during embryogenesis.

Epigenotype-phenotype relationships in patients with features of Beckwith-Wiedemann syndrome. *E.L. Niemitz^{2,3}, A.P. Feinberg^{2,4}, S. Brandenburg², V. Kanchananakhin¹, M.R. DeBaun¹.* 1) Division of Pediatric Hematology-Oncology, Department of Pediatrics, Washington University School of Medicine, St. Louis MO; 2) Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore MD; 3) Predoctoral Program in Human Genetics, Johns Hopkins University School of Medicine, Baltimore MD; 4) Departments of Medicine, Oncology, and Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore MD.

Beckwith-Wiedemann syndrome (BWS) is characterized by prenatal overgrowth, macroglossia, midline abdominal-wall defects, and predisposition to Wilms and other embryonal tumors. BWS serves as a model for birth defects and cancer caused by imprinted genes, i.e. those preferentially expressed from a specific parental allele. The most common constitutional abnormalities in BWS are epigenetic, involving abnormal methylation of either *H19* or *LIT1*, which encode untranslated RNAs on 11p15. We have attempted to determine the relationship between epigenotype and phenotype in BWS by analyzing a cohort of BWS patients for these molecular abnormalities. The odds ratio for cancer in children with altered *H19* methylation was 6.0 (95% CI 1.3-26.5), while midline abdominal wall defects and macrosomia were significantly associated with altered *LIT1* methylation. These results define an epigenotype-phenotype relationship in BWS, in which distinct epigenetic alterations are strongly associated with cancer risk and specific birth defects. These epigenetic alterations were in marked contrast to patients with isolated hemihypertrophy. We are also investigating the relationship between multiple phenotypic and epigenotypic features of BWS in families.

Imprinting analysis of genes flanking *MEST* at 7q32. T. Yamada^{1,4}, K. Mitsuya², T. Kayashima^{1,4}, K. Yamasaki^{1,4}, T. Ohta^{3,4}, T. Kishino^{3,4}, M. Oshimura², N. Niikawa^{1,4}. 1) Department of Human Genetics, Nagasaki University School of Medicine, Nagasaki, Japan; 2) Molecular and Cell Genetics, School of Life science, Tottori Univ., Yonago, Japan; 3) Nagasaki University Gene Research Center, Nagasaki, Japan; 4) CREST, JST, Kawaguchi, Japan.

MEST located at 7q32 is the paternally expressed gene in the mesodermal derivatives at early embryonic stages and imprinted in both human and mouse fetal tissues. Since some imprinted genes are clustered to form an imprinted domain in a chromosomal region, it is reasonable to presume that there exists another imprinted domain at 7q32, where *MEST* and its flanking genes are involved in genomic imprinting. We previously demonstrated that *COPG2* and *TSGA14*, both neighboring on *MEST*, showed biallelic expression, whereas *COPG2IT1* (*CIT1*) was paternally expressed and *CPA3* maternally expressed. Here we report the imprinting status of nine other genes located to the *MEST*-flanking 7q32 region. Allelic expression of these genes was examined by RT-PCR in fetal samples heterozygous for nucleotide polymorphisms and also analyzed using mouse A9 somatic cell hybrids. All genes but *TSGA13* which are spanning an approximately 1.2-Mb region were evaluated. Of the eleven genes in this region, three (*MEST*, *COPG2IT1* and *CPA3*) showed imprinted expression but the remaining eight genes, i.e., *NRF1*, *UBE2H*, *HSPC216*, *KIAA0265*, *CPA2*, *CPA1*, *TSGA14* and *COPG2*, revealed not imprinted. These findings that most genes in the vicinity of *MEST* are not imprinted may suggest that the *MEST*-flanking 7q32 region is not strictly controlled as an imprinted domain.

Relative levels of the *UBE3A* sense transcript and the IC-*SNURF-SNRPN* sense/*UBE3A* antisense transcript in blood and brain. *M. Runte*¹, *P.M. Kroisel*², *C. Lich*¹, *B. Horsthemke*¹, *K. Buiting*¹. 1) Institut für Humangenetik, Universitätsklinikum Essen, Essen, Germany; 2) Institut für Medizinische Biologie und Humangenetik, Universität Graz, Graz, Austria.

The *UBE3A* gene, which is affected in Angelman syndrome, is expressed in brain from the maternal allele only. In contrast to other imprinted genes, monoallelic expression of *UBE3A* does not appear to be regulated by a differentially methylated region. It has been suggested that the paternal allele is silenced through a paternally-expressed *SNURF-SNRPN* sense/*UBE3A* antisense transcript, which starts at the imprinting center (IC). To exclude other start sites we performed real-time RT-PCR on blood RNA of IC deletion carriers. As expected, in two IC deletion patients with Prader-Willi syndrome, who have the deletion on the paternal chromosome, the deletion results in the complete loss of expression of this transcript, whereas in their fathers, who have the deletion on the maternal chromosome, an IC-deletion has no effect. To find out if and how the IC-*SNURF-SNRPN* sense/*UBE3A* antisense transcript might regulate imprinted expression of *UBE3A* in brain, we have compared the levels of both transcripts in human blood and brain by RT-PCR. In blood, where *UBE3A* is biallelically expressed, we found that the *SNURF-SNRPN* exons 1-2 and 57-58 are expressed at a level similar to *UBE3A*, but the 3'-, antisense part of the transcript was barely detectable. In fetal brain, where the paternal *UBE3A* allele is silenced, the 3' end is present at a level similar to the maternal *UBE3A* sense transcript. These findings are compatible with a model in which imprinted *UBE3A* expression is regulated by alternative 3' ends or splice variants of the *SNURF-SNRPN* sense/*UBE3A* antisense transcript.

Reactivation of imprinted genes in a Prader-Willi syndrome mouse model. *K. Takano*¹, *M. Takahashi*¹, *A. Sudo*¹, *T. Wada*¹, *T. Ohta*², *R.D. Nicholls*², *S. Saitoh*¹. 1) Dept. Pediatrics, Hokkaido Univ., Sapporo, Japan; 2) Dept. Psychiatry, Univ. Pennsylvania, Philadelphia, PA, USA.

Prader-Willi syndrome (PWS) is associated with the loss of paternal gene expression in 15q11-q13. The maternal alleles are present but inactivated by genomic imprinting, so reactivation of these genes could ameliorate PWS clinical symptoms. Previously, we demonstrated that a key imprinted gene, *SNURF-SNRPN*, could be reactivated by treatment with a methylation inhibitor, 5-azadeoxycytidine (5-aza-dC), but not by the histone deacetylase inhibitor trichostatin A (TSA). To further investigate a potential therapeutic role for these drugs, we have now used as a model mice that carry a deletion in 7C that is homologous to human 15q11-q13. We established fibroblasts from PWS and Angelman syndrome model mice (with the deletion on the paternal or maternal chromosome, respectively), and wild type controls. These cells were treated with 5-aza-dC, TSA, or both. Expression of 3 paternally-expressed genes (*Snurf-Snrpn*, *Ndn*, *Mkrn3*) and a control gene (*Gapdh*) was examined by RT-PCR, while histone acetylation at 5' CpG islands was examined by chromatin immunoprecipitation with anti-acetylated histone H3 and H4. As in human cells, the imprinted genes were reactivated by 5-azadC but not by TSA alone. While *Snurf-Snrpn* was only slightly reactivated by 5-azadC, *Ndn* and *Mkrn3* were readily reactivated. The level of reactivation correlated with increased levels of histone acetylation. These findings indicate that imprinted genes in mouse cells can be reactivated by a DNA methylation inhibitor, histone acetylation correlates with reactivation, and the reactivation pattern may differ between human and mice. Studies with PWS mice will examine further the potential use and limitations of these therapeutic agents.

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Role of histone methyltransferase G9a in H3 Lys9 methylation and CpG methylation of the Prader-Willi imprinting center. Z. Xin¹, M. Tachibana², Y. Shinkai², J. Wagstaff¹. 1) University of Virginia School of Medicine, Charlottesville, VA; 2) Kyoto University, Kyoto, Japan.

Imprinted genes in the Angelman/Prader-Willi (AS/PWS) region are subject to long-range regulation by cis-acting sequences known as imprinting centers (ICs). The mechanisms by which these ICs exert their effects is unknown. The PWS-IC, which overlaps the major promoter of SNRPN, is required for establishment and maintenance of the paternal pattern of gene expression of the AS/PWS region. The PWS-IC shows CpG methylation specific for the maternal chromosome, which has been hypothesized to be the gametic imprint for the AS/PWS region. In the mouse, parent-specific CpG methylation of this region is established during gametogenesis. However, a recent study has shown that the PWS-IC is completely unmethylated in human oocytes (O. El-Maarri et al., *Nature Genet.* 27:341-344). We have proposed histone H3 methylation on Lys9 as a candidate gametic imprint for the AS/PWS region (Z. Xin et al., *Am. J. Hum. Genet.* 69:1389-1394), with CpG methylation of the IC and of other promoter regions occurring as a consequence of the primary histone-based imprint. Here we show that both histone H3 Lys9 methylation and CpG methylation of the PWS-IC in mouse ES cells are dependent on the function of the G9a histone H3 Lys9/Lys27methyltransferase. Our results demonstrate a role for histone H3 methylation in the maintenance of parent-specific CpG methylation of imprinting regulatory regions. These observations suggest a possible role of histone methylation in establishment of imprints during gametogenesis.

Analysis of *cis*-acting elements in the 5' region of the *SNRPN* gene. *S. Rodriguez-Jato*¹, *D.J. Driscoll*^{2,3}, *T.P. Yang*^{1,3}. 1) Dept. of Biochem & Molec Biol; 2) Dept. of Pediatrics; 3) Center for Mammalian Genetics, University of Florida College of Medicine, Gainesville, FL.

The Prader-Willi/Angelman syndrome (PWS/AS) region on chromosome 15q11-q13 includes a cluster of imprinted genes that are coordinately regulated by an imprinting center (IC) that spans the 5' region of the *SNRPN* gene. The IC has a bipartite structure where the PWS-IC is postulated to enhance the transcription of paternally-inherited alleles, while the AS-IC is postulated to negatively regulate the PWS-IC. We are interested in analyzing *cis*-acting elements within the IC that may mediate IC function and/or *SNRPN* promoter activity. The PWS-IC contains a major nuclease hypersensitive site (HS) associated with the *SNRPN* promoter region on the paternally-inherited allele. A 2nd HS on the paternal allele is found adjacent to the PWS-IC in the 1st intron of the *SNRPN* gene. We have used transient expression assays to examine *cis*-acting elements within these HS sites. We generated constructs in which a reporter gene was driven by segments of the *SNRPN* 5' flanking region in human neuroblastoma and fibrosarcoma cells. These studies define the minimal *SNRPN* promoter and suggest the existence of a negative regulatory element in the promoter region. Introducing sequences from the intronic HS into *SNRPN* expression constructs reveals a position dependent and orientation independent activator function in the HS. These intronic sequences show the same effect on the SV40 and imprinted *MKRN3* promoters. These data suggest the intronic HS may play a role in the postulated transcriptional activation function of the PWS-IC. Previous analysis of *cis*-acting elements in the *SNRPN* promoter region by in vivo footprinting identified 4 transcription factor binding sites on the paternal allele. To examine the function of these sites, we mutated each one and determined the effects on promoter activity in transient expression assays. We found that two of the footprinted sites affect *SNRPN* promoter function, while the two other sites do not. These data suggest the 2 latter sites may have an alternate function, such as a role in IC function and/or establishing chromatin structure of the paternal *SNRPN* allele.

Chloride channel mutations in proximal myotonic myopathy: implications for disease modulating effects. *S.F. Ursu, N.H. Mao, F. Lehmann-Horn, K. Jurkat-Rott.* Applied Physiology, University of Ulm, ULM, Germany.

Proximal myotonic myopathy (PROMM) is a multisystemic disorder with similarity to myotonic dystrophy (DM) but without the abnormal (CTG) expansion in the DMPK (myotonic dystrophy protein kinase) gene. The inheritance is autosomal dominant and the clinical feature includes myotonia, proximal weakness and cataracts. Recently, the disease causing mutation, a CCTG expansion, located in intron 1 of the zinc finger protein 9 (ZNF9) gene was identified. We tested 32 families diagnosed as PROMM for the presence of expanded alleles by indirect linkage test (using the CL3N58 microsatellite marker). Because of the presence of the CCTG repeats in the marker, the mutant allele is too large to be amplified. Therefore this marker shows an abnormal segregation pattern: all affected individuals are hemizygous and seem to have only one allele (the normal one), and affected children (hemizygous also) appear not to inherit an allele from their affected parent. Using this indirect test we determined that 19 of 32 tested families (59,37%) presented this segregation pattern. The affected members of these families did not have the CTG expansion in DMPK gene either thus demonstrating genetic heterogeneity. It is known that non-dystrophic myotonia in humans may be caused by mutation in the gene coding for skeletal muscle chloride channel (CLCN1). Using multiplex allele specific PCR, all these 19 families were tested for mutation in CLCN1. We found that 6 of the 19 positive families (31,57%) have the R894X. Because the frequency of this mutation in the normal population is 1%, this is unlikely to be coincidental. Only one PROMM affected individual was homozygous for R894X and therefore additionally has Becker myotonia. All other affected families members with R894X mutation in CLCN1 were heterozygous and showed no other known CLCN1 mutation. We suggest that presence of CLCN1 mutations in PROMM may enhance myotonia, and that therefore these people seek medical aid, which ultimately leads to more frequent diagnosis of patients. Our observations imply disease-causing mutation in genes, other than the causative one to modulate the PROMM phenotype.

Analysis of six candidate genes for autism on 7q. *AHMM. Huq, H. Zhong, R. Nabi, F. Serajee.* Departments of Pediatrics and Neurology, Wayne State University, Detroit, MI.

Autism is a neurodevelopmental disorder with a complex genetic etiology. Several independent genome scans have revealed excess allele sharing in an overlapping 40 cM region of 7q21-34. However some studies show an additional linkage peak further distally on 7q. We studied single nucleotide polymorphisms (SNPs) in DLX6, PCLO, metabotropic glutamate receptor 3 (GRM3) and metabotropic glutamate receptor 8 (GRM8) genes on 7q21-32 and vasoactive intestinal peptide receptor type 2 (VIPR2) and engrailed homologue 2 (EN-2) genes on 7q36.3 for evidence of association and linkage to autism using 196 multiplex autistic disorder families. Transmission disequilibrium testing did not show any association between VIPR2, EN-2, DLX6, PCLO and GRM3 genes and autistic disorder. There was evidence for association with stereotypic behavior and parent-of-origin effects for age-of-onset for phrase-speech for a SNP in the GRM8 gene. Affected sib pair studies using SNP genotype data and publicly available genotype and phenotype data from the same Autism Genetics Resource Exchange families showed increased identity by descent sharing and a maximal multipoint NPL (GENEHUNTER) score of 2.2 on 7q36 at the VIPR2 locus. When stratified according to language development, families in which all affected sibs have useful phrase speech contributed to linkage at both 7q21 and 7q36, but the families in which at least one sib has no useful phrase speech contributed to linkage at 7q36 only. Quantitative trait linkage analysis revealed evidence of linkage to 7q21, 7q31 and 7q36 regions. Using nonparametric multipoint linkage analysis, the strongest quantitative trait locus evidence was for useful phrase speech level between markers D7S1830 and D7S2204 at 7q21 ($Z = 3.97$, one sided $p = 0.00003$) and for stereotypic behavior at GRM8 locus at 7q31 ($Z = 3.94$, one sided $p = 0.00004$). Taken together, these data may suggest the presence of more than one autism susceptibility locus at chromosome 7q.

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Analysis of five serotonin system genes in autism by association and linkage. *R. Nabi, H. Zhong, D.C. Chugani, F.J. Serajee, AHMM. Huq.* Departments of Pediatrics and Neurology, Wayne state University, Detroit, MI.

Although abnormalities of serotonin system have been described in autism, genetic analyses of serotonin transporter, receptors and related genes have given conflicting results. We have investigated single nucleotide polymorphisms in serotonin 1B receptor (HTR1B), serotonin 2A receptor (HTR2A), monoamine oxidase A (MAOA), tryptophan hydroxylase (TPH) and tryptophan 2, 3 dioxygenase (TDO2) genes for linkage and association in autistic disorder. 196 multiplex autistic disorder families from Autism Genetic resource Exchange (AGRE) were tested using transmission disequilibrium and two-point affected sib pair linkage analysis. We found no evidence of association with intragenic markers in HTR1B ($c2 = 0.26$; $p = 0.61$), HTR2A ($c2 = 1.45$; $p = 0.23$), MAOA ($c2 = 0.08$; $p = 0.78$) and TPH ($c2 = 0.03$; $p = 0.87$) with autism. In addition, there was also no linkage or association between language and stereotypic behavior quantitative traits in autism and single nucleotide polymorphisms. TDT studies of a SNP in TDO2 provided suggestive evidence of association ($c2 = 5$; $p = 0.02$) in an initial set of 44 autistic disorder families ascertained by referral from the local clinicians. However, additional studies in 196 multiplex AGRE families did not demonstrate any evidence of association. In conclusion, our studies suggest that these five serotonin system genes are unlikely to play a major role in autism.

Association of the metabotropic glutamate receptor 8 gene at 7q31 with quantitative traits in autism. *H. Zhong, F.J. Serajee, R. Nabi, AHMM. Huq.* Departments of Pediatrics and Neurology, Wayne State University, Detroit, MI.

Several independent genome scans have revealed linkage of autism to the chromosome 7q21-32 region. We sequenced the promoter region and exons of the metabotropic glutamate receptor 8 (GRM8) gene at 7q31 in 40 individuals and investigated several intragenic single nucleotide polymorphism (SNPs) in the GRM8 gene for evidence of association and linkage to autism using 196 multiplex autistic disorder families. Two point nonparametric linkage studies with GENEHUNTER demonstrated evidence for linkage of an intron 8 SNP to stereotypic behavior (Z score 2.95), useful phrase-speech level (Z score 1.3) and age-of-onset for phrase-speech (Z score 1.6). Multipoint linkage studies revealed a nonparametric Z score of 3.94 (one sided $p = 0.00004$) at the intron 8 SNP for stereotyped behavior. Variance component analysis using MERLIN also revealed evidence of linkage to stereotyped behavior ($p = 0.003$). Using the QTDT package, there was nominally significant association of the intron 8 SNP with stereotyped behavior ($p = 0.017$). However, when paternal and maternal alleles were modeled separately, there was highly significant association of the intron 8 SNP with age of onset for phrase-speech in autistic subjects. To further explore the parent-of-origin effects, we performed RTPCR studies which demonstrated biallelic expression of the major splice forms of the GRM8 gene in hippocampus and cerebral cortex. Transmission disequilibrium testing (TDT) did not show any association between the intron 8 SNP and autistic disorder ($p = 0.14$). However, when age-of-onset of phrase-speech was used to determine the affection status, TDT analysis showed evidence of association during maternal transmission ($c^2 = 4.35$, $p = 0.02$), but not during paternal transmission ($c^2 = 0.01$, $p = 0.92$). In addition, there was evidence of linkage disequilibrium ($p = 0.03$) between a haplotype of intron 8 and exon 10 SNP and autistic disorder. Taken together, these data suggest the presence of a susceptibility mutation in the GRM8 or a nearby gene.

Natural selection may have impact to maintain linkage disequilibrium at interleukin-4/ interleukin-13 region on chromosome 5q31-33. *T. Sakagami*^{1,2}, *T. Nakajima*¹, *N. Jinnai*¹, *T. Hasegawa*², *E. Suzuki*², *F. Gejyo*², *I. Inoue*¹. 1) Division of Genetic Diagnosis, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan; 2) Division of Respiratory Medicine, Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan.

Linkage disequilibrium (LD) has recently become the focus of interest in the mapping of complex disease loci through genotype-phenotype association studies. To better understand the pattern of LD in the human genome, the refined structure of LD in a 25.6 kb region covering the entire human interleukin 13 gene (IL13) and interleukin 4 gene (IL4) was evaluated among Africans, Caucasians, and Japanese, based on 73 single nucleotide polymorphisms (SNPs). IL13 and IL4 locate adjacently on chromosome 5q31-33 where susceptibility loci for several allergic diseases were reported. Two near-perfect LD blocks, one spanning 0.8 kb in IL13 and the other spanning at least 20 kb in IL4, were identified in Caucasians and Japanese, but not in Africans. Within LD blocks at IL13 and IL4, very limited number of distinct haplotypes, which were shared between Caucasian and Japanese, were counted. Neutrality test was performed on the regions inside and outside the LD blocks suggesting that a selective sweep occurred at the region inside the LD blocks while the neutrality was maintained outside the LD blocks. The results allowed us to infer that a selective sweep could be one of the factors to generate and maintain LD block.

UABSNP58 on human chromosome 20 associated with insulin resistance. *S.M. Sell¹, T. Kekes-Szabo¹, D. Chen¹, C. Song¹, H. Chen¹, L. Baier², M. Traurig², R. Hanson².* 1) Dept Nutrition Sci, 346 Webb, Univ Alabama at Birmingham, Birmingham, AL; 2) NIDDK, Phoenix, AZ.

Several groups have reported linkage to type 2 diabetes on human chromosome 20q. This region has also been linked to other obesity-related traits, suggesting that a gene (or genes) on chromosome 20q may contribute to the obesity-related and pre-diabetic trait of insulin resistance. Molecular genetic and homozygosity mapping studies carried out in our laboratory have identified a single nucleotide polymorphism, UABSNP58, that is associated with increased risk for obesity-induced diabetes in subjects of English ancestry. The Pima Indians of Arizona have a high incidence of obesity and diabetes. Metabolic predictors of diabetes and obesity, such as insulin resistance, have been extensively characterized in subjects from this population. Therefore, UABSNP58 was genotyped in more than 1100 DNA samples from Pima Indians. This SNP was not associated with type 2 diabetes in these Pima subjects (N=739 diabetic and 433 non-diabetic). However, in 223 non-diabetic, full-heritage Pima Indians who had undergone a hyperinsulinemic, euglycemic clamp to assess insulin action, UABSNP58 was highly associated with insulin resistance at the high dose of insulin (8.06 ± 0.28 mg/min.kg. vs 9.32 ± 0.38 mg/min.kg; $p=0.0005$) after adjusting for age, sex, % fat and family membership). This association with insulin resistance supports a role for UABSNP58 (and/or an allele (s) in linkage disequilibrium with UABSNP58) in genetic susceptibility for insulin resistance, and consequently an increased risk for type 2 diabetes in some populations. The nearest known gene to UABSNP58 is the Kreisler mouse maf-related leucine zipper homolog (KRML1) a bZIP transcription factor. This gene is expressed in skeletal muscle and adipose tissue (Sell, unpublished) and other bZIP transcription factors have been implicated in diabetes-susceptibility; consequently, we consider KRML1 to be a good positional candidate gene. However, there may also be additional SNPs in linkage disequilibrium with UABSNP58 that contribute to insulin resistance. This work was supported by funds from NIH grants DK056336 and HD040788.

No association between the APOE gene and Autistic Disorder. *K.L. Raiford¹, Y. Shao¹, I.C. Allen¹, M.M. Menold¹, M.L. Cuccaro¹, J.R. Gilbert¹, H.H. Wright², R.A. Abramson², G. Worley¹, J.M. Vance¹, M.A. Pericak-Vance¹.* 1) Duke University Medical Center, Durham, NC; 2) University of South Carolina, Columbia, SC.

Autistic Disorder (AutD) is a neurodevelopmental disorder characterized by stereotypic and repetitive behavior and interests, together with social and communicative deficiencies. Peak linkage/association scores on several genomic screens indicate the presence of an autistic disorder susceptibility locus on chromosome 19p13.2 - q13.4. The apolipoprotein E (APOE) gene on chromosome 19 encodes for a protein, apoE, whose different isoforms (-E2, -E3, -E4) influence neuronal growth, participates in lipid transport and metabolism, repair, growth, and maintenance of axons and myelin during neuronal development. The APOE gene competes with the reelin gene for VLDL/APOER2 receptor binding. A previous report found evidence for an association between AutD and the reelin gene. In addition, it has been suggested that the APOE-2 allele has a protective effect against infertility and miscarriage in AutD families. Based on these data we examined APOE as a candidate gene in AutD. We tested for genetic association using family-based association methods in a data set of 163 multiplex Duke and AGRE AutD families (2 or more AutD affecteds per family) and 159 singleton Duke AutD families (1 AutD affected per family). We also tested APOE promoter SNPs 113cg, 219gt, 427ct, 491at, and 5361ct. We saw no significant evidence that AutD is associated with the APOE gene (PDT $p=0.93$; TRANSMIT $p=0.45$) or the APOE promoter SNPs.

Investigation of the association of candidate genes with Attention Deficit Hyperactivity Disorder. *J.L. Stone¹, V. Kustanovich¹, M. Ogdie¹, M. Yang², E. Paja³, J. McCracken³, S. Smalley^{2,3}, S. Nelson^{1,2}.* 1) Dept Human Gen, Gonda, #5554, Univ California, Los Angeles, Los Angeles, CA; 2) Center for Neurobehavioral Genetics, Univ California, Los Angeles, Los Angeles, CA; 3) Department of Psychiatry and Biobehavioral Sciences, Univ California, Los Angeles, Los Angeles, CA.

Attention Deficit Hyperactivity Disorder (ADHD) is the most common neuropsychiatric disorder among school-aged children. ADHD is a complex disorder with a considerable etiology attributed to genetics. A recently completed genome scan (Fischer, 2002) indicated several regions of suggestive linkage to the disease. Several reasonable candidate genes are located under the regions of suggestive linkage: DRD5 (4q16.1), CALCYON (10q26.3), HTR1A (5q12.3), HTR1B (6q14.1), HTR1E (6q15), HTR5A (7q36.3), SLC6A4 (17q11.2). CALCYON and DRD5 are involved in the dopamine pathway, while the other six genes are all involved in the serotonin pathway. A sample of 204 multiplex families containing over 270 affected sib pairs from the Los Angeles basin was genotyped for a series of microsatellite markers and single nucleotide polymorphisms (SNPs). Evidence of linkage was tested by applying the transmission disequilibrium test (TDT) to either genotypic or inferred haplotype data.

Genome Scan of Schizophrenia, Bipolar Disorder and Psychosis in Portuguese families. C.N. Pato^{1,2}, P. Sklar³, M. Daly³, A. Verner³, A. Kirby³, T. Hudson³, H. Medeiros¹, C.P. Morley¹, A. Macedo², A. Dourado², I. Coelho², J. Valente², M.J. Soares², C.P. Ferreira^{1,2}, C. Carvalho¹, J.L. Kennedy^{1,4}, M.H. Azevedo², E. Lander³, M.T. Pato^{1,2}. 1) Psychiatry and VA, SUNY-Upstate, Syracuse, NY; 2) Universidade de Coimbra, Coimbra, Portugal; 3) Whitehead Institute Center for Genome Research, Cambridge, Mass, USA; 4) Clarke Division, Centre for Addiction and Mental Health, Toronto, Canada.

Purpose: We have performed an initial genome-wide scan on 360 individuals from 67 multiplex Portuguese families primarily from island populations. The advantages of using Azorean and Madeiran families for mapping include their relatively recent settlement in the 1500s, geographic isolation and lack of significant inward migration. **Methods:** We genotyped 391 microsatellite markers for an approximately 10 cM resolution. Markers had an average heterozygosity of 75%. Families were analyzed in three diagnostic categories: schizophrenia (DSM-IV diagnoses of schizophrenia and schizoaffective disorder depressed), bipolar disorder (DSM-IV bipolar disorder or schizoaffective manic) or psychosis (lifetime history of psychotic episode). Multipoint nonparametric linkage analysis using GENEHUNTER software was performed. **Results:** In the schizophrenia families NPL scores of greater than 1.7 were obtained on chromosomes 1, 5, and 8. The regions on 1 and 5 also had NPL scores greater than 1.8 when all individuals with psychosis were considered regardless of disease phenotype. An additional chromosomal region was identified for psychosis on chromosome 11. In families with bipolar disorder, there were 6 chromosomal regions with NPL scores greater than 2.0 on chromosomes 2, 6, 9, 18, 19 and 20. We are currently fine mapping these regions in an additional 35 families to replicate the previous findings and to characterize candidate genes in the region. Our observation of evidence for linkage common to schizophrenia and psychosis may indicate that genes in these regions play a role in both phenotypes, while other evidence for linkage appears to be specific for bipolar disorder and schizophrenia and not the common phenotype psychosis.

Candidate gene analysis in autism. *K.S. Beyer*¹, *S.M. Klauck*¹, *F. Blasi*², *E. Bacchelli*², *A. Benner*³, *S. Epp*¹, *F. Poustka*⁴, *E. Maestrini*², *A. Poustka*¹, *The International Molecular Genetic Study of Autism Consortium (IMGSAC)*⁵. 1) Molecular Genome Analysis, Deutsches Krebsforschungszentrum, Heidelberg, Germany; 2) Department of Biology, University of Bologna, Italy; 3) Biostatistics, Deutsches Krebsforschungszentrum, Heidelberg, Germany; 4) Department of Child and Adolescent Psychiatry, J.W. Goethe University, Frankfurt, Germany; 5) <http://www.well.ox.ac.uk/~maestrin/iat.html>.

Autism is a severe developmental disorder with an onset in early childhood, characterized by marked social deficits, deviant language and a restricted range of stereotyped repetitive behaviors. A genetic etiology is strongly indicated by twin and family studies. The most recent genome screen completed by IMGSAC on a total data set of 152 sib-pairs revealed susceptibility loci on 12 chromosomes, with the highest results on chromosomes 2q (MLS 3.74 at D2S2188) and 7q (MLS 3.37 at D7S477). Using the carefully diagnosed sample of more than 140 trios recruited in Germany and Austria, analysis of positional and functional candidate genes especially from the region on chromosome 7q under the peak of linkage was performed. Coding regions of candidate genes (e.g. WNT2) were systematically screened for non-synonymous variants using SSCP or DHPLC (denaturing high performance liquid chromatography) followed by direct sequencing. Polymorphisms identified in these studies as well as known variants can be used in association studies. So far no strong contribution to the genetic etiology of autism of any of the genes under investigation was found in the whole patient sample. Due to the partial overlap of phenotypical symptoms between autism and Rett syndrome, the coding region of the methyl-CpG binding protein 2 (MECP2) gene on Xq28 has been systematically screened for mutations in 152 autistic patients from 134 German families and 50 unrelated patients from the IMGSAC affected relative-pair sample. Three novel non-synonymous variants cosegregating with autism in the available family members were identified. Taking into account the large size of our sample, we conclude that mutations in the coding region of MECP2 do not play a major role in autism susceptibility.

A third pass genome screen in panic disorder. *A.J. Fyer^{1,2}, S.P. Hamilton^{1,2}, M. Durner^{1,2}, G.A. Heiman¹, D.F. Klein^{1,2}, S.E. Hodge^{1,2,3}, J.A. Knowles^{1,2}, M.M. Weissman^{1,2}.* 1) Department of Psychiatry, Columbia University College of Physicians and Surgeons, New York, NY; 2) New York State Psychiatric Institute, New York, NY; 3) Department of Biostatistics, Columbia University, New York, NY.

Panic disorder (PD) is a common, disabling illness characterized by recurrent, unexpected episodes of intense anxiety, subsequent worry and phobic avoidance. Family, twin and segregation analytic data indicate that PD is moderately heritable (35-49%), but do not support a specific mode of transmission. We report a third stage genome screen from the Columbia Collaborative Panic Project. Two previous scans conducted in the first 23 and 34 families in this sample had their maximum lod scores (1.71 and 2.45 respectively) in the region of chromosome 7p15. In the current scan 391 microsatellite marker loci (average spacing 9cM) were genotyped in 1005 individuals from 120 multiplex panic disorder pedigrees. Data were analyzed under two modes of inheritance (dominant, recessive) using three pre-designated diagnostic thresholds for panic disorder (Broad, Intermediate, Narrow).

Results: Three loci (on chromosomes 2, 9, and 17) show maximal single locus lod scores >3.3 ; and one (chromosome 15) a score of 2.9. Convergent results from multipoint and NPL analyses were found for three (2,9,15) of these four regions. We also found continued evidence for linkage between a region on chromosome 13 and a previously hypothesized panic disorder "syndrome" related to bladder/ renal problems. Two markers in the identified area of chromosome 13 had single locus scores over 3 in the subset of syndrome families. There was decreased evidence for linkage in the previous region of interest on chromosome 7p (max lod <1).

These data indicate several chromosomal regions that may be important in the genetic contribution to panic disorder and are consistent with clinical data in suggesting a complex genetic architecture for this disorder.

The frequency of NOD2 risk alleles in the International IBD Genetic Consortium (IBDGC) Families. J.A. Cavanaugh and The Inflammatory Bowel Disease Genetics Consortium. The Canberra Hospital, Australia.

In 2001, three alleles in the NOD2 gene on chromosome 16 associated with susceptibility to Crohns disease (CD) were reported. Functional and positional evidence supports this association. The IBDGC now reports on an analysis of the NOD2 variants [Arg702Trp (C/T), Gly908Arg (G/C) and 980fs981(-/C)] in families previously used to validate linkage to the IBD1 locus with an NPL score of 5.79.

Table 1 shows details of allele frequencies in the probands (defined as the first affected sibling per family) for each disease grouping (CD, UC or mixed). As expected from the linkage evidence demonstrating that IBD1 locus is a CD locus, the highest allele frequencies are observed in CD probands. While individual centers have already produced some support for the identification of NOD2 risk alleles as the causative alleles in the IBD1 gene, this is the first comparison of the frequencies of these alleles on a population-by-population basis, and permits the accumulation of large numbers for disease group comparisons. Preliminary linkage analysis suggests that some evidence for linkage remains after the effect of NOD2 is removed.

Table 1

	ALL	CD	UC	Mixed
# probands	648	383	126	139
Arg702Trp	.07	.10	.02	.06
Gly908Arg	.04	.06	.02	.02
980fs981	.08	.10	.02	.06

Genetics of antinuclear antibody repertoires in Systemic Lupus Erythematosus. *M. Barreto¹, C. Fesel¹, F. Fontes¹, R. Andreia², J.F. Viana², F. Crespo², C. Vasconcelos², A.M. Vicente¹, C. Ferreira².* 1) Instituto Gulbenkian Ciencia, Oeiras, Portugal; 2) Associação dos Doentes com Lupus, Lisboa, Portugal.

SLE is a multifactorial disorder with heterogeneous presentation, in which genetic susceptibility plays a major role. The common denominator among SLE patients is IgG autoantibody production against a wide variety of nuclear antigens (antinuclear antibodies, ANA). The main objective of this study is the identification and characterization of genetic susceptibility factors for SLE. The strategy used is the identification of lupus-associated traits that are genetically less complex, and therefore more amenable to genetic mapping. For this purpose, and given the systematic presence of ANA in patients, we have analyzed serum IgG antibody repertoires against a HEp2 nuclear extract in a sample of 16 multiplex families, including 26 patients and 72 unaffected relatives, and 46 adult healthy controls. A quantitative immunoblotting procedure and multiparametric analysis show a distinct pattern of autoantibody reactivities in patients, compared with controls ($p=0.0092$ for principal component analysis (PCA) factor 1 and $p=0.042$ for PCA factor 2), with two major antigenic bands contributing for these PCA factors. Correlation coefficients for relative pairs were established for PCA factor 1 ($r_{M/F}=-0.419$, $r_{M/D}=0.348$, $r_{F/S}=0.428$, $r_{MA/N}=0.532$) and for PCA factor 2 ($r_{M/F}=0.127$, $r_{M/D}=0.128$, $r_{F/S}=0.653$, $r_{MA/N}=0.331$), indicating the presence of a genetic component for the autoantibody reactivity patterns in these SLE multiplex families. Heritability was estimated at 6% for PCA factor 1 and 21% for PCA factor 2. The present results confirm the specificity of ANA in SLE patients. Most important, the multiparametric analysis uncovered shared autoantibody reactivity patterns among relatives, indicating that this may constitute a genetic trait appropriate for mapping. Detailed segregation analysis of the patterns of autoantibody reactivities in a larger sample is required, paving the way for genetic mapping of this SLE associated phenotype.

Susceptibility loci for atopic dermatitis on chromosomes 3, 13, 15, 17, and 18 in a Swedish population. M.

Bradley^{1,2}, *C. Söderhäll*², *H. Luhtman*^{2,3}, *C-F. Wahlgren*², *I. Kockum*², *M. Nordenskjöld*². 1) Department of Dermatology and Venereology; 2) Department of Molecular Medicine, Karolinska Institutet, Stockholm, Sweden; 3) Present address: Department of Endocrinology, Wallenberg Laboratory, University Hospital MAS, Malmö, Sweden.

Atopic dermatitis is a hereditary, pruritic, inflammatory, and chronic skin disease that typically presents in the early childhood and may continue or recur later. The etiology of atopic dermatitis is unknown, but several lines of evidence indicate that it is a multifactorial disorder caused by the combined influence of genetic and environmental factors, even though the relative contributions of genes and environment are not known. To identify important loci that contribute to the development of atopic dermatitis, we conducted a genome-wide linkage analysis with 367 microsatellite markers, using a non-parametric affected relative pair method in 109 pedigrees. Three qualitative phenotypes and one semi-quantitative phenotype were studied. For the phenotype atopic dermatitis linkage to chromosome region 3p24-22 was found. For another phenotype, atopic dermatitis combined with raised allergen-specific IgE levels, suggestive linkage was found to chromosome region 18q21. For the semi-quantitative phenotype, severity score of atopic dermatitis, suggestive linkage was found to chromosome regions 3q14, 13q14, 15q14-15 and 17q21. Identifying chromosome regions linked to susceptibility genes for atopic dermatitis provides a platform from which the search for atopic dermatitis genes can proceed.

Candidate Gene Studies for Linkage and Genetic Association to Spina Bifida in Hispanic and non-Hispanic White Populations in North America. *T.J. Kirkpatrick¹, K.-S. Au¹, P.X. Tran¹, S. Bassett¹, S.H. Blanton², H. Northrup^{1,3}*. 1) Dept of Pediatrics, Univ Texas Medical School, Houston, TX; 2) Dept of Pediatrics, Univ of Virginia, Charlottesville, VA; 3) Shriners Hospital for Children, Houston, TX.

Spina bifida (SB) is one of the most common severe birth defects in North America with an incidence of 1/2,000. SB occurs secondary to a combination of genetic and environmental factors with heritability estimated at 60%. Due to the lack of clear-cut inheritance and paucity of families with multiple affected members, we have chosen to search for genetic linkage and/or association by candidate gene studies of simplex families (child-mother-father trios and child-parent pairs). Our sample set consists of 260 complete trios (130 Hispanic, 120 non-Hispanic White and 10 Other), 250 mother-child pairs (150 Hispanic, 80 non-Hispanic White and 20 Other), 25 father-child pairs (15 Hispanic, 8 non-Hispanic White and 2 Other). Our recent studies have focused on candidate genes in the following categories: growth factors and growth factor receptors (IGF1, IGF2, NGFb, FGF1, FGF3, TGFb and IGF2R), transcription factors (BRCA1, ZIC3 and CREBBP) and proto-oncogenes (WNT1 and BCL2). The candidate genes were selected based on function in development or animal models. Markers (di-, tri- and tetranucleotide repeats) from within or immediately adjacent to (within 5-10 kb) each gene were tested in the entire sample set. For some candidate genes, multiple markers were tested. Results are currently being analyzed by transmission disequilibrium testing (TDT) for linkage and association for the entire sample set, by ethnicity and by level of defect.

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Efficient fine-mapping of a type 2 diabetes locus on chromosome 20 through SNP typing on DNA pools. *P.E. Hollstein¹, K. Silander¹, L.J. Scott², T.E. Fingerlin², P.S. Chines¹, K.L. Mohlke¹, N. Narisu¹, M.R. Erdos¹, M. Li², T.T. Valle³, R.N. Bergman⁴, J. Tuomilehto³, M. Boehnke², F.S. Collins¹.* 1) National Human Genome Research Inst., NIH, Bethesda, MD; 2) Dept. of Biostatistics, U. of Michigan, Ann Arbor, MI; 3) Dept. of Epidemiology and Health Promotion, National Public Health Institute, Helsinki, Finland; 4) Dept. of Physiology and Biophysics, Keck School of Medicine, U. of Southern California, Los Angeles, CA.

The Finland-United States Investigation of NIDDM Genetics (FUSION) study aims to positionally clone variants that predispose to type 2 diabetes. A genome scan of 478 Finnish affected sibling pair families yielded the highest LOD score of 2.20 on chromosome 20 at 69.5 cM and a 1-LOD support interval of 11 cM (6.9 Mb). To fine-map this interval, we have used primer extension and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry to genotype 220 SNPs on 3 DNA pools from diabetic cases (n=197, 250, and 499) and 2 DNA pools of control subjects (n=182 and 228). The average marker density was 30 kb and all SNPs had allele frequency >10%. For each pool and SNP, we calculated allele frequencies and standard deviation (SD) (average SD=1.93%) based on 4 PCR reactions spotted 4 times each (n=16). SNPs were prioritized based on frequency differences, SD, and sample size. We obtained allele frequency differences >5% between case and control pools in 23 SNPs, including a cluster of 6 SNPs spanning 102 kb. We typed individuals on these 6 SNPs and observed allele frequency differences consistent with those seen in pools. For one of the SNPs, we confirmed an allele frequency difference of 11% between cases with evidence of linkage to 20q13 and unaffected controls. For two other SNPs 500 kb apart we confirmed 10% allele frequency differences between 20q13-linked cases and unaffected controls, with p-values of 0.0001 and 0.0005. We are now typing additional SNPs on pools over the 6.9 Mb locus to reduce our current intermarker distances down to 15-20 kb and we will continue confirming significant results by typing individuals. This strategy should ultimately allow precise identification of the susceptibility variant(s) for type 2 diabetes on chromosome 20q.

Genetic linkage and association of haplotypes at the serotonin transporter locus in a rigid-compulsions subphenotype of autism. *J.L. McCauley¹, M. Dowd³, L.M. Olson¹, T. Amin¹, R.D. Blakely², S.E. Folstein³, J.L. Haines^{1,2}, J.S. Sutcliffe^{1,2}.* 1) Program in Human Genetics, Department Molecular Physiology & Biophysics; 2) Center for Molecular Neuroscience, Vanderbilt University, Nashville, TN; 3) Department of Psychiatry, Tufts Univ. and New England Medical Center.

Autism is a complex genetic neurodevelopmental disorder in which affected individuals display deficits in language and social relationships and patterns of compulsive and stereotyped behaviors and rigidity. While genome-wide linkage screens have identified a number of regions, locus heterogeneity complicates efforts to identify these genes for autism. To identify genetically more homogeneous family subsets, we performed a factor analysis of the Autism Diagnostic Interview to define subphenotypes in autism. Linkage analysis in a subset of families with compulsive behaviors and rigidity identified significantly increased evidence for linkage on chromosome 17q11.2, with a maximum HLOD score of 2.82 at D17S1294. The serotonin transporter locus (SLC6A4) maps nearby and is proposed as a candidate in autism, based upon the effect of selective serotonin reuptake inhibitors in treating compulsions and anxiety in autism and findings of hyperserotonemia in a subset of people with autism. We genotyped single nucleotide polymorphisms (SNPs) across the 25-kb transcriptional unit in 149 multiplex families. Three of these SNPs were recently proposed to associate with autism (Kim et al, *Mol Psychiatry* 7: 278, 2002). Analysis of individual markers for transmission disequilibrium (TD) revealed allelic effects for one SNP in intron 4 ($P < 0.02$), and analysis of multi-SNP haplotypes using TRANSMIT showed significant TD for two haplotypes defined by three other SNPs ($P < 0.02$ and $P < 0.04$). Examination of the rigid-compulsive subset revealed a similar result for the single SNP but substantially increased TD for the same haplotypes ($P < 0.002$ and $P < 0.005$). These data support a role for SLC6A4 in the etiology of autism and related traits, demonstrate the utility of subphenotypes for defining more homogeneous datasets for genetic studies and show that analysis of haplotypes is crucial for detection of risk alleles in complex genetic disease.

Dense linkage disequilibrium mapping in the 15q11-q13 maternal expression domain yields evidence for association in autism. *E.L. Nurmi¹, L.M. Olson², M.M. Jacobs¹, T. Amin², J.L. McCauley², A.Y. Lam², S.E. Folstein³, J.L. Haines^{1,2}, J.S. Sutcliffe^{1,2}.* 1) Center for Molecular Neuroscience; 2) Program in Human Genetics, Department of Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN; 3) Department of Psychiatry, Tufts University, New England Medical Center, Boston, MA.

Autism is a complex yet strongly genetic neurodevelopmental disorder. Chromosome 15q11-q13 has been proposed to harbor a gene contributing to autism susceptibility based on (i) maternal chromosomal duplications seen in autism and (ii) evidence for linkage and linkage disequilibrium (LD) at 15q markers in chromosomally normal autism families. In order to investigate and localize a potential susceptibility variant, we developed a dense single nucleotide polymorphism (SNP) map of the maternal expression domain in proximal 15q, consisting of 29 SNPs spanning the two known imprinted, maternally-expressed genes in the interval, UBE3A and ATP10C, and putative imprinting control regions. With a marker coverage of 1/10 kb in coding regions and 1/15 kb in large 5' introns, this map was employed to dissect LD in autism families. Four SNPs within ATP10C demonstrated evidence for preferential allelic transmission to affected offspring ($P < 0.05$). All four lie within islands of sequence homology between human and mouse genomes that may be directly functional or may instead belong to an ancestral haplotype containing a functional susceptibility allele. The region was further explored for recombination hot spots and haplotype blocks to evaluate haplotype transmission in our families. Interpretation of these data will require replication across data sets, evaluation of potential functional effects of associated alleles, and a thorough assessment of haplotype transmission within ATP10C and neighboring genes. Nevertheless, these findings are consistent with the presence of an autism susceptibility locus in 15q11-q13.

Analysis of nine candidate genes for autism on chromosome 2q. *E. Maestrini*¹, *E. Bacchelli*¹, *F. Blasi*¹, *M. Biondolillo*¹, *A.J. Bailey*², *A.P. Monaco*³, *International Molecular Genetic Study of Autism Consortium (IMGSAC)*⁴. 1) Dept. Biology, Univ of Bologna, Italy; 2) CSGDP, Inst. Psychiatry, London, UK; 3) WTCHG, University of Oxford, Oxford, UK; 4) <http://www.well.ox.ac.uk/~maestrin/iat.html>.

In order to identify susceptibility genes for autism, the IMGSAC performed a whole genome screen for linkage in affected sib-pair families and identified the strongest result on chromosome 2q31-q33. Supporting evidence for the presence of an autism susceptibility locus on 2q is provided by the convergence of linkage findings from independent genome scans. We are screening candidate genes mapping to this region, to identify variants that may contribute to the aetiology of autism. To date, we have analysed nine genes: DLX1, DLX2, TBR1, CHN1, GAD1, CREB2/ATF2, cAMP-GEFII, NEUROD1, and HOXD1. All coding regions and putative functional sequences have been screened by DHPLC and sequencing in a subset of 48 cases from IMGSAC families that are contributing to the linkage peak on 2q. Non-synonymous variants identified in each gene have been investigated in a control sample. In addition, the most frequent SNPs detected through the screening have been tested for association in the whole IMGSAC sample. The cAMP-GEFII gene provided the most interesting results. Four non-synonymous variants have been identified in this gene, each present in both affected sibs of a family and absent in 192 controls. The cAMP-GEFII gene was further investigated in a second set of 48 IMGSAC cases. One of the previously identified variants was found in an additional sib-pair, but no further non-synonymous variants were identified. Furthermore, analysis of 8 intragenic SNPs did not provide significant evidence for association with autism. The significance of these variants is unclear; their low frequency in IMGSAC families does not account for the relatively strong linkage at the 2q locus, and suggests that they are likely to represent very rare polymorphism rather than aetiological variants. In conclusion, no evidence was found that any of the above candidate genes strongly contributes to autism susceptibility. Further investigation is needed to clarify if variants in cAMP-GEFII may play a role in a small subset of cases.

Linkage Disequilibrium Mapping Provides Further Evidence for a Gene for Reading Disability on Chromosome

6P21.3-22. *M.C. O'Donovan¹, D. Turic¹, L. Robinson¹, M. Duke¹, D.W. Morris¹, V. Webb¹, M. Hamshere¹, A. Grierson¹, N. Williams¹, M. Van den Bree¹, R. Chowdhury², P. McGuffin³, J. Stevenson⁴, M. Krawczak^{1,5}, M.J. Owen¹, J. Williams¹.* 1) Psychological Medicine, UWCM, Cardiff, UK; 2) Dept of Neuroscience and Cognitive Dev, Babraham Institute, Cambridge, UK; 3) Social, Genetic and Developmental Psychiatry, IOP, Denmark Hill, London, UK; 4) Centre for Research into Psychological Development, Univ of Southampton, UK; 5) Department of Medical Genetics, UWCM, Cardiff, UK.

Linkage disequilibrium (LD) mapping was used to follow up reports of linkage between reading disability (RD) and a 18 cM region of chromosome 6p21.3-22. Using a two-stage approach, we tested for association between RD and 21 microsatellite markers in two independent samples of 101 (Stage 1) and 77 (Stage 2) parent/proband trios in which RD was rigorously defined. The most significant replicated associations were observed between combinations of markers D6S109/422/1665 (Stage 1 $p = 0.002$, (adjusted for multiple testing); Stage 2 $p = 0.0001$) and D6S506/1029/1660 (Stage 1 $p = 0.02$ (adjusted), Stage 2 $p = 0.0001$). The only two-marker association observed in both samples was with D6S422/1665 ($p = 0.01$; $p = 0.04$). No single marker showed replicated association but D6S506 produced values of $p = 0.01$ & 0.08 which were significant when combined ($p = 0.02$). We observed weaker and less consistent evidence of association in a region of confirmed linkage to RD in previous studies. The most consistently significant haplotypic association D6S109/422/1665, showed association with single-word reading, spelling, phonological awareness, phonological decoding, orthographic accuracy and random automatised naming, but not with vocabulary or Attention Deficit Hyperactivity Disorder. Our findings strongly support the presence of a gene contributing to RD in a region of chromosome 6 between markers D6S109 and D6S1260, but do not rule out the presence of a gene between D6S1556 and MOG.

Association analysis of ISL1 and PAX4 in Swedish Type 1 Diabetes families. *P. Holm¹, B. Rydlander¹, H. Luthman^{1,2}, I. Kockum¹.* 1) Dept Molec Med, Clin Genetics, Karolinska Hosp, Stockholm, Sweden; 2) Department of Endocrinology, Lund University, Malmö, Sweden.

Type 1 diabetes mellitus (T1DM) is an autoimmune disease caused by destruction of the pancreatic insulin-producing beta cells. ISL1 is a transcription factor, which together with other transcription factors regulates the development and function of the pancreatic islet cells. ISL1 have previously been studied for linkage and association to T2DM, in this study we are investigating the role of ISL1 in T1DM. Sequencing of the ISL1 gene was performed in 12 patients and 4 healthy controls to identify DNA variations, four of the SNPs found in the gene were then analysed in Swedish multiplex T1DM families. The variations found in the ISL1 gene were all in non-coding regions. A tendency to association with T1DM could be shown for the ISL1 SNP 47G allele. In order to investigate this association further, we investigated familial association by analysing transmission to affected individuals for haplotypes of SNP markers. The identification of this suggestive association to ISL1 markers will now make it possible to use case-control materials to confirm the involvement of ISL1 in T1DM susceptibility. Since interaction between several susceptibility genes are likely to occur in T1DM, we analysed interaction between the ISL1 locus and other chromosomal regions harbouring other genes involved in the development and maintenance of the endocrine pancreas. The closest microsatellite marker to each gene was selected for analysis of interaction with ISL1. We could demonstrate interaction between the ISL1 locus on chromosome 5 and chromosome 7q32, a region encoding the PAX4 gene. PAX4 is a transcription factor that plays an important role in the development and differentiation of the islet b- and d-cells. A binding site for ISL1 is present in the promoter region of the PAX4 gene, and it has also been shown that PAX4 has an inhibitory effect of insulin gene transcription by binding to its promoter. Three SNPs were chosen in the PAX4 gene for further studies and showed association to T1DM in the Swedish families when calculating PDT for haplotypes consisting of all three SNPs within the PAX4 gene.

Do circadian disturbances mediate the pathogenesis of Bipolar I Disorder? *H. Mansour¹, J.A. Wood¹, L. Brar¹, T. Monk¹, E. Frank¹, D. Kupfer¹, V.L. Nimgaonkar^{1,2}*. 1) Dept of Psychiatry, Western Psychiatric Institute and Clinic, Univ Pittsburgh, Pittsburgh, PA; 2) Dept of Human Genetics, Univ Pittsburgh, Pittsburgh, PA.

We are evaluating circadian function as well as variations in circadian genes in order to examine their relevance to bipolar disorder I (BPD1). We are using two relevant quantitative measures: the composite scale (CS), a measure of morningness-eveningness and the social rhythm metric (SRM), which estimates social cues that influence circadian function. We have recruited 100 BD1 cases (DSM IV criteria), their available parents and 349 community-based controls. For comparison, 70 patients with schizophrenia (SZ) and their parents are available. Analysis of variation suggested significant age by group interactions (sum of squares 1268.4; $F = 5.978$, 4 df, $p < 0.0001$). Trends for significant case-parent correlations were also observed (BD1: $r = 0.257$, 35 case-parent pairs, $p = 0.136$; SZ: $r = 0.177$, $p = 0.296$, 37 pairs). We are presently investigating SRM scores individually and in conjunction with CS. In parallel, we are also investigating polymorphisms in genes that critically influence circadian function. Haplotype-based Transmission Disequilibrium Tests (TDT), as well as case-control analyses did not reveal significant associations at the CLOCK locus using BD1 as the phenotype. We are currently investigating four other candidate genes: Timeless (Tim), Cryptochrome 1 (Cry1), Period 1 (Per1) and Bmal1 (ARNTL).

***RHD* maternal-fetal genotype incompatibility increases schizophrenia susceptibility.** C.G. Palmer¹, J.A. Turunen², J.S. Sinsheimer¹, S. Minassian¹, J. Lönnqvist², L. Peltonen¹, J.A. Woodward¹. 1) University of California, Los Angeles, CA, USA; 2) National Public Health Institute, Helsinki, Finland.

Fetal events and obstetric complications are associated with schizophrenia. We report the results of a family-based candidate gene study that assesses the role of maternal-fetal genotype incompatibility at the *RHD* locus in schizophrenia. We adapted the case-parent-trio log-linear modeling approach to develop a maternal-fetal genotype incompatibility test, performed using MISMATCH, that is sensitive to the effects of *RHD* maternal-fetal genotype incompatibility and distinguishes these effects from a high risk allele at or near the *RHD* locus. 88 case-parent trios, 72 case-mother pairs, and 21 case-father pairs were genotyped at the *RHD* locus. Of the 181 cases, 62% were male, 81% were second or later born, 80% were diagnosed with schizophrenia, 14% with schizoaffective psychosis disorder, and 6% with a schizophrenia spectrum diagnosis. Only 3 cases were born after prophylaxis against maternal isoimmunization had become common practice in Finland. Of the 160 cases for which maternal *RHD* genotypes were available, 7.5% had genotype D/d (Rh+) when the maternal genotype was d/d (Rh-). There was significant evidence for an *RHD* maternal-fetal genotype incompatibility ($c^2 = 3.52$, 1df, [1-sided] $p = .03$), and the value of the incompatibility parameter was estimated at 2.53. There was no evidence to support linkage/association at or near the *RHD* locus with schizophrenia ($c^2 = .90$, 2 df, [2-sided] $p = 0.64$). There also was no evidence to support the role of maternal genotype effect alone ($c^2 = .38$, 2 df [2-sided] $p = 0.83$). The *RHD* locus increases risk for schizophrenia through a maternal-fetal genotype incompatibility mechanism that enhances risk of an adverse prenatal environment, i.e., Rh incompatibility, rather than via linkage/association with a high-risk allele at or near the *RHD* locus. This biological mechanism is consistent with the teratogenic antibody hypothesis of interference with normal fetal neurodevelopment. This is the first candidate gene study to explicitly test for, and provide evidence in support of, a maternal-fetal genotype incompatibility mechanism in schizophrenia.

Identification of a High Risk Haplotype for the Dystrobrevin Binding Protein 1 (DTNBP1) Gene in the Irish Study of High Density Schizophrenia Families. *E. Van den Oord¹, P. Sullivan¹, D. Walsh², A. O'Neill³, K. Kendler¹, B. Riley¹.* 1) Psychiatry, Virginia Commonwealth Univ., Richmond, VA; 2) Psychiatry, The Queens University, Belfast, Northern Ireland; 3) The Health Research Board, Dublin, Ireland.

A recent report suggested significant associations between several SNPs in a previously unknown EST cluster with schizophrenia (Straub et al. 2002). The cluster was identified as the human dystrobrevin binding protein 1 gene (DTNBP1) by sequence database comparisons and homology with mouse DTNBP1 (Jiang et al. 2002). However, the linkage disequilibrium (LD) among the SNPs in DTNBP1 as well as the pattern of significant SNP-schizophrenia association was complex. This raised several questions such as the number of susceptibility alleles that may be involved and the size of the region where the actual disease mutation(s) could be located. To address the questions we performed different single marker tests on the 12 previously studied and 2 new SNPs in DTNBP1 that were re-scored using an improved procedure, and performed a variety of haplotype analyses. The sample consisted of 268 Irish multiplex families selected for high density of schizophrenia. Results suggested a simple structure where the LD in the target region could be explained by 6 haplotypes that together accounted for 96 percent of haplotype diversity in the whole sample. From these six, a single high risk haplotype was identified that showed a significant association with schizophrenia and explained the pattern of significant findings in the analyses with individual markers. This haplotype was 30 kb long, had a large effect, could be measured with two tag SNPs only, had a frequency of 6% in our sample, seemed to be of relatively recent origin in evolutionary terms, and was equally distributed over Ireland. Implications of these finding for follow up and replication studies are discussed.

A genome-wide search for celiac disease loci in Dutch affected siblings shows significant linkage to chromosome 19. *M.J. van Belzen¹, A.F.J. Bardoel¹, W. van der Hoeven², C.J.J. Mulder³, J. Meijer³, L.A. Sandkuij¹, P.L. Pearson¹, R.H.J. Houwen², C. Wijmenga¹.* 1) Medical Genetics, UMCU, Utrecht, The Netherlands; 2) Pediatrics, UMCU, Utrecht, The Netherlands; 3) Hepatogastroenterology, Rijnstate Hospital, Arnhem, The Netherlands.

Celiac disease (CD) is a common multifactorial disorder that affects the small intestine. Its estimated prevalence is 0.5% in Western populations. Ingestion of dietary gluten from wheat, barley and rye results in villous atrophy of the duodenum with hyperplastic crypts and infiltration of the gut epithelium with lymphocytes. So far, the only established locus for CD is the HLA region. Most CD patients express DQ2, almost all the others are DQ8. Concordance between HLA-DQ identical siblings is only 30%. Furthermore, the population frequency of DQ2 is 25%, which is much higher than the prevalence of CD. This indicates that non-HLA genes are also involved in the etiology of CD. Therefore, we conducted a genome-wide screen to identify new, non-HLA loci. Strict criteria were used for inclusion of affected sibpairs, since they were diagnosed in over 50 different hospitals. All initial biopsy specimens were recovered and evaluated by our own pathologist to insure homogeneity of the diagnosis. Over 300 microsatellite markers were typed in 67 families with a total of 74 Marsh III sibpairs. Multipoint linkage analysis was performed by the mapmaker/sibs program. We identified strong linkage to chromosome 19, with a LOD score of 3. Finemapping of this region and addition of 12 more sibpairs resulted in statistically significant linkage with a LOD score of 3.8. Six other loci with a LOD score >1 were found, including the HLA region with a LOD score of 6. Interestingly, the telomeric region on chromosome 5q, in which some evidence for linkage was found in three other CD genome screens, showed a LOD score of 1.2. More markers and families are currently typed for the candidate regions to evaluate the significance of our findings.

Fine mapping of Alzheimers disease linkage region on chromosome 10. *D. Turic¹, M. Dunstan¹, F. Rice¹, S. McIlroy², D. Craig², P. Passmore², A. Myers³, S. Lovestone⁴, J. Powell⁴, J. Hardy⁵, A. Goate⁶, M. Liddell¹, L. Jones¹, M. O'Donovan¹, M.J. Owen¹, J. Williams¹.* 1) Psychological Medicine, UWCM, Cardiff, United Kingdom; 2) Queens University Belfast, Belfast, United Kingdom; 3) Washington University, St Louis, United States; 4) Institute of Psychiatry, London, United Kingdom; 5) National Institute on Aging, Bethesda, United States; 6) Department of Psychiatry, St Louis, United States.

In a recent two-stage genome-wide screen for late onset AD we observed significant linkage to chromosome 10. The linked region is large spanning approximately 44cM from markers D10S1426 to D10S2327. We used linkage disequilibrium mapping with 44 microsatellite markers to further refine the MaxLOD-1 region (~22cM). A three stage design strategy was employed: (1) First, markers were tested for association in pooled DNA samples comprising of 128 AD cases, 57 affected sibling pairs (NINCDS-ARDB probable/definite, late onset AD) and 103 age matched controls sampled from the UK population; (2) In stage two markers reaching p-value of $p < 0.1$ using the DAIP pooled analysis method, were genotyped individually; (3) Finally, significant markers from the second stage ($p < 0.05$) were individually typed on an independent replication sample of 229 late onset AD cases and 230 controls sampled from Belfast, N.Ireland. Out of 44 markers four were positive at stage one: D10S567, D10S1790, D10S1762 and D10S1647, two of these, D10S1790 and D10S1647, remained significant after individual typing. Marker D10S1647 showed association with AD in the replication sample. However, this relationship did not show further evidence of replication in set of 92 AD cases and 94 controls sampled from the USA population (St Louis).

A Genome Search for Type 2 Diabetes Susceptibility Genes in West Africans. C. Rotimi¹, G. Chen¹, J. Zhou¹, D. Parish-Gause¹, H. Daniel¹, Y. Chen¹, A. Amoah², J. Acheampong³, K. Agyenim-Boateng³, B. Eghan Jr.³, J. Oli⁴, G. Okafor⁴, P. Furbert-harris¹, B. Osotimehin⁵, F. Abbiyesuku⁵, T. Johnson⁶, O. Fasimade⁶, R. Kittles¹, G. Dunston¹, F. Collins⁷ and Africa America Diabetes Mellitus (AADM) Study. 1) Natl Human Genome Ctr, Howard Univ, Washington, DC; 2) University of Ghana, Accra; 3) University of Science and Technology, Ghana; 4) University of Nigeria, Enugu; 5) UCH, Ibadan, Nigeria; 6) University of Lagos, Nigeria; 7) NHGRI, NIH, Bethesda.

A genomic scan was conducted to identify loci linked to type 2 diabetes in 691 individuals from 343 families from two West African ancestral populations of African-Americans (Ghana: Accra and Kumasi; Nigeria: Enugu, Ibadan and Lagos). A total of 390 STR markers were scored by the Center for Inherited Disease Research (CIDR) for an average spacing of 9 cM with no gaps greater than 20 cM. Multipoint linkage analysis was conducted using GENEHUNTER-Plus and ASM programs. Evidences of linkage were observed on chromosomes 1, 4, 10, 12, 17, 19 and 20 (p-value < 0.05). The highest LOD score of 2.63 was observed on chromosome 20 between markers D20S480 and D20S171 followed by chromosomes 12 (between markers D12S2070 and D12S395) and 19 (D19S714) both with a score of 1.92 and 1.81 respectively. Ordered subset analyses based on sibships with extreme mean values of diabetes related quantitative traits identified the sets of families which contributed disproportionately to the peaks in chromosomes 12, 19 and 20. We also conducted multipoint variance components linkage analyses on related diabetes traits with the GENEHUNTER2 program. The strong signal on chromosome 20, which lies in the region implicated as harboring diabetes and obesity susceptibility genes in previous studies reinforces and provides additional support that chromosome 20, may indeed contain susceptibility loci for diabetes. Linkage evidence provided in this study is particularly important given the evolutionary history of these West African populations and their well documented historic contribution to the forced migration of Africans to the Americans; African Americans have one of the highest rates of diabetes in the world.

Genome Search for Genes Underlying Susceptibility to Insulin Resistance Syndrome in Type 2 Diabetes Patients from West Africa. J. Zhou¹, G. Chen¹, A. Doumatey¹, P. Furbert-Harris¹, A. Amoah², J. Acheampong³, J. Oli⁴, B. Osotimehin⁵, T. Johnson⁶, K. Agyenim-Boateng³, G. Okafor⁴, F. Abbiyesuku⁵, O. Fasimade⁶, B. Eghan Jr.³, H. Daniel¹, Y. Chen¹, D. Parish-Gause¹, G. Dunston¹, F. Collins⁷, C. Rotimi¹ and Africa America Diabetes Mellitus (AADM) Study. 1) National Human Genome Center, Howard University, Washington, DC; 2) University of Ghana, Accra; 3) University of Science and Technology, Ghana; 4) University of Nigeria, Enugu; 5) UCH, Ibadan, Nigeria; 6) University of Lagos, Nigeria; 7) NHGRI, NIH, Bethesda.

Insulin Resistance Syndrome (IRS) represents a cluster of metabolic abnormalities including obesity, glucose intolerance, hypertension, and dyslipidemia. We use Principal Component factor analysis (PCFA) to evaluate linkage and association between genetic factors and IRS related phenotypes including BMI, Fat Mass, %Fat Mass, Blood Pressure, Insulin, Glucose, HDL, triglycerides and LEPTIN in our diabetes cohort from Nigeria and Ghana. A total of 856 individuals from 357 sib-pairs were included in these analyses. The PCFA yielded three principal factors: factor 1 (BMI [0.88], FM [0.93], PFM [0.91] and LOG LEP [0.76]) with eigenvalue of 3.4; factor 2 (LOG TG [0.89] and HDL [-0.90]) with eigenvalue of 1.8; factor 3 (SBP [0.86] and DBP [0.86]) with eigenvalue of 1.66. Factors 1, 2, and 3 respectively explained 34%, 17% and 16% for a total of 67% of observed total variance. Factors 2 and 3 were significantly higher in the cases than controls. Using GENEHUNTER, we performed multipoint variance component genome search for regions linked to the 3 complex phenotypes. All analyses were adjusted for gender, age and age. Strongest evidence of linkage was found for factor 2 (lipid traits; LOD =3.2, P = 0.00007; between D3S2427 and D3S1262); followed by factor 1 (adiposity traits; LOD =2.1, P = 0.0009; between D4S1644 and D4S1625) and factor 3 (BP traits; LOD = 2.1, p=0.001). Using QTDT, we demonstrated evidence of association in these linkage regions. In conclusion, we developed complex IRS traits, estimated heritability and found suggestive evidence of linkage and association on chromosome 3q27-28, 4q31 and 16p11.

Genome-wide Association of Diabetes Traits in the Old Order Amish. *P.L. St Jean¹, W.-C. Hsueh², H. Sakul³, M.W. Wagner¹, D.K. Burns¹, T.I. Pollin⁴, B.D. Mitchell⁴, A.R. Shuldiner⁴.* 1) GlaxoSmithKline, RTP, NC; 2) UCSF School of Medicine, San Francisco, CA; 3) Pfizer Global R&D, Groton, CT; 4) University of Maryland School of Medicine, Baltimore, MD.

We have performed genome-wide tests of association for diabetes traits in a genetically homogenous population, the Old Order Amish using 412 autosomal STR markers. The average intermarker density was 9.3 cM although chromosomes 1 & 14 were more densely typed due to prior evidence of linkage to diabetes. Our previous work showed that LD between STRs in the Amish averages between 5-10 cM making a genome-wide association with these markers a reasonable undertaking. Two traits were examined: Type 2 diabetes (DM) and a broader phenotype of DM or impaired glucose homeostasis (DM/IH). The DM data consisted of 201 subjects (59 with DM) in 25 pedigrees. The DM/IH data consisted of 374 subjects (125 with DM/IH) in 48 pedigrees. Association within families was assessed using Transmit 2.4 aggregating alleles occurring at a frequency $\geq 5\%$. Both global and allelic tests were examined. No tests were significant at a $P \leq 0.00012$ using a Bonferroni adjusted p-value. When a less stringent cut-off of $P \leq 0.005$ was employed the following associations were revealed. Chr 1: DM/IH and 3 STRs (D1S252, D1S534, D1S1187) between 154-172 cM. DM was also associated with D1S1187. Chr 5: DM/IH and D5S416 at 27.2 cM and D5S400 at 181.7 cM. Chr12: DM/IH and D12S352 at 0 cM. Chr 15: DM/IH and D15S117 at 48.7 cM. Chr18: DM/IH and 2 STRS - D18S464 at 31.6 cM and D18S478 at 50.2 cM. Two of these regions have provided evidence of linkage to diabetes traits in the Amish. The area on chromosome 1 shows evidence of linkage to both DM & DM/IH in the Amish. Other populations such as the Pima, Utah, UK and French Caucasians have reported linkage to diabetes traits in this region. D18S464 is within 3 cM of a peak linked to diabetes in obese Nordic families. In the Amish we have observed LOD scores between 1.4-3.0 for 2-hour glucose and HbA1C between 9-45 cM on chromosome 18. In summary, our association results in conjunction with linkage results provide supporting evidence for diabetes loci on chromosomes 1 and 18.

Use of pooled DNA in case-control studies: How precision of allele frequency estimation affects efficiency. *L.J. Scott¹, C. Li¹, T.E. Fingerlan¹, A.U. Jackson¹, K.L. Mohlke², K. Silander², F.S. Collins², M. Boehnke¹.* 1) Dept. Biostatistics, Univ Michigan, Ann Arbor, MI; 2) NHGRI, NIH, Bethesda, MD.

Fine-mapping of complex trait susceptibility regions requires genotyping of large numbers of SNPs. Constructing DNA pools from cases and controls can drastically reduce the required genotyping given that the method accurately and precisely estimates pooled allele frequencies. We examined the precision and number of replicates required to limit the number of false positives (FPs) due to the use of pools. For reference we typed 16 SNPs (previously typed on individuals) in 3 pools (n= 499, 228 and 182) using mass spectrometry (16 replicates per pool, SD = 2.1 + 1.0%). For our calculations we assumed pools sizes that would have 80% power, if typed in individuals, to detect 5 and 10% allele frequency differences in the population at a 5% significance level, i.e. 5% are FPs. Setting power at 80% in pools, we examined FPs for a variety of SDs and number of replicates. These results were virtually unaffected the control allele frequency (50% in Table). A FP of 10% in pools means that half of the significant pooling results would not be significant if individuals were genotyped (under the null). The number of replicates needed to maintain FP rates <=10% increased from 4 for 1% SD to 4-16 for 2% SD to 8- >16 for 3% SD. These calculations depend on the absence of systematic bias in the pooled measurements and thus it is important to assess both SD and potential bias in allele frequency estimates.

SD (%)	1	1	1	2	2	2	3	3	3
Replicates (n)	4	8	16	4	8	16	4	8	16
5% dif (%FP)	8	6	6	19	11	8	36	20	12
10% dif (%FP)	6	6	5	8	6	6	12	8	7

Screening for chromosomal aberrations in mentally retarded patients using MAPH. *J.T. Den Dunnen, M. Kriek, S. White, M. Kalf, S. Uitte De Willige, G.J.B. Van Ommen, M.H. Breuning.* Human and Clinical Genetics, Leiden Univ Medical Ctr, Leiden, Nederland.

Submicroscopic chromosomal rearrangements are believed to play an important role in the etiology of mental retardation. To facilitate the detection of these rearrangements we have implemented Multiplex Amplifiable Probe Hybridisation. MAPH is a PCR-based method to simultaneously determine the copy number of a large set, currently up to 80, of different chromosomal loci. The probes, usually exons from candidate genes, are individually cloned such that all can be amplified using one pair of primers. To detect copy number changes, the probes are hybridised to genomic DNA immobilized on nylon filters. After stringent washing, hybridised probes are recovered off the filters, quantitatively amplified and analysed using a 96 capillary sequencer. The yield, represented by peak height and area, is determined for each probe. Changes in probe yield correspond to changes in copy number of the sequence analysed, i.e. a deletion or duplication. The advantage of MAPH compared to other techniques, including SKY, FISH and array-CGH, is that the resolution of detection is limited only by the size of the probes used (100-500 bp). In addition, the technique facilitates the parallel screening of several tens of patients at many different loci in one experiment. We have generated a probe set covering 300 different genomic loci, including subtelomeric regions, regions involved in microdeletion syndromes and X-linked mental retardation and a set of genes evenly spread through out the rest of the genome. Using this probe set we have screened 200 mentally retarded patients. Preliminary results include rearrangements in both subtelomeric and pericentromeric regions, as well in several chromosomes. As reported before, we detected more aberrations in patients with mental retardation and dysmorphic features compared to patients with mental retardation only (for the subtelomeric probe set 13.3% and 7.3% respectively). Our long term goal is to develop a 3000-loci probe set covering the entire human genome with a one Mb spacing and to implement it for molecular karyotyping in a diagnostic setting.

Gene-gene Interaction in Drug-induced Weight Gain on Schizophrenic Patients. *T. Lan^{1,2}, Y. Wong¹, J. Chan¹, H. Hsieh¹, J. Sou^{1,3}, T. Hu¹, J. Chiu^{1,3,4}.* 1) Department of Psychiatry, Yu-Li Veterans Hospital, Hualien, Taiwan; 2) Institute of Genetics, School of Life Medicine, National Yang-Ming University, Taipei, Taiwan; 3) Institute of Public Health, Faculty of Medicine, National Yang-Ming University, Taipei, Taiwan; 4) Section of Psychiatry, Faculty of Medicine, National Yang-Ming University, Taipei, Taiwan.

Drug-induced weight gain has been considered as an emerging adverse side effect of atypical antipsychotics such as clozapine in treating schizophrenic patients. We recruited 93 schizophrenic patients treated with clozapine for 14 months after all consent form being completed. The body weight change between endpoint and baseline as the outcome, we genotyped several candidate genes related to obesity, including 5HT2A, ADRA1A, ADRA2A promotor, ADRB3, and TNF-alpha, for each participants. After adjusting for age, sex, baseline body weight, and TNF-alpha genotypes; a statistically significant interaction ($p = 0.030$) between ADRA1A genotypes and ADRA2A promotor genotypes was found on the weight gain in these schizophrenic patients. This suggests that schizophrenic patients with some specific combination of different genotypes of candidate genes for obesity have a higher tendency to gain weight compared to those without such characteristics.

Quantification of heteroplasmic A3243G mutation of mitochondrial DNA by real-time PCR. *R. Bai, L. Wong.* Inst for Mol & Hum Genetics, Georgetown Univ. Med. Ctr., Washington, DC.

It is well-known that the tRNA leu (UUR) point mutation at position 3243 in mitochondrial DNA (mtDNA) is associated with the syndrome of mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) and is also found in patients with maternally-inherited diabetes and deafness syndrome (MIDD) and progressive kidney disease. In order to correlate disease expression with mutation load, it is necessary to quantitate the percentage of the mutant mtDNA. The most commonly used method for quantification of A3243G mutant is PCR/RFLP that requires either radioactive material or DNA intercalating dye. This method usually can not provide accurate quantification if the mutant load is low. To overcome this difficulty, we established a real-time PCR assay to quantify the A3243G mutant mtDNA. The copy number of mtDNA was calculated from standard curve using TaqMan probes 6FAM-TTACCGGGCTCTGCCATCT-TAMRA and 6FAM-TTACCGGGCCCTGCCATCT-TAMRA for normal and mutant respectively. The percentage of mutant mtDNA copy was calculated from the normal and mutant mtDNA. Twenty A3243G positive samples detected by allele specific oligonucleotide (ASO) and quantified by PCR/RFLP method were studied. Experimental samples with different percentage of mutant A3243G mtDNA were generated by mixing the normal DNA sample with DNA containing 92% mutant DNA (detected by PCR/RFLP). Each measurement was performed in duplicate and each sample was repeated in at least two different runs. In addition, four control samples containing 75%, 25%, 10% and 1% of mutant were included in each run to ensure that the results from different test runs are comparable. Our results showed high reproducibility with a coefficient of variation (CV) of <5%. The results from the six experimental samples are very consistent with the percentages expected. The results from the 20 clinical samples are also consistent with the previous measurement, however, the real-time quantitative PCR method is much more sensitive in detecting low percentage (<1%) mutant. These results indicate that real-time PCR assay is a fast, sensitive, and reliable method for quantification of mutant mtDNA.

Methylation profiling of CpG islands spanning the MHC class II region and HLA-B in Ankylosing Spondylitis cases and controls. *D.T. Akey¹, J.M. Akey¹, J.D. Reveille², L. Jin¹.* 1) Department of Environmental Health, Center for Genome Information, University of Cincinnati, Cincinnati, OH; 2) Department of Rheumatology, University of Texas-Houston Health Science Center, Houston, TX.

Ankylosing spondylitis (AS) is an autoimmune disease with a complex pattern of inheritance. Although 90% of individuals with AS are HLA-B27 positive, the exact mechanisms contributing to disease susceptibility remain unknown. Recently, we have performed statistical analyses of markers in the HLA class II region in 180 AS sib-pairs that suggests the presence of a second AS susceptibility gene, which is subject to a parent-of-origin effect (GeneHunter Imprinting, maximum parent of origin LOD score = 1.62 for marker D6S1583). One potential mechanism that may result in a parent-of-origin effect is DNA methylation, which is the covalent modification of cytosine to 5-methylcytosine at CpG dinucleotides. To investigate the hypothesis that DNA methylation status may be a disease mechanism in the pathogenesis of AS, we have begun to systematically examine the methylation state of CpG islands in the HLA class II region as well as the class I gene, HLA-B. Specifically, melting curve based methylation specific PCR (McMSP), melting curve based COBRA (McCOBRA), and bisulfite sequencing are being used to determine the methylation status of 12 CpG islands spanning 722 Kb of the MHC class II region and a CpG island located within the promoter and first three exons of HLA-B in 21 AS pedigrees (96 individuals). Our study design allows for both family and population based analyses. These data will allow us to: 1) compare DNA methylation status of AS susceptibility genes in cases versus controls, 2) establish if methylation status of these loci are stably transmitted from parent to offspring, and 3) examine whether there are allelic differences in methylation status for HLA-B and class II genes in both normal and affected individuals. In summary, we will present the first large scale analysis of DNA methylation in the class II region and determine its role in AS susceptibility.

Normal Methylation Pattern At The 5' SNRPN Locus In Patients From The South Carolina Autism Project. S.A. Copeland-Yates^{1,2}, R.J. Schroer¹, C. Skinner¹, R.C. Michaelis¹. 1) Dept Biochemistry & Genetics, Clemson Univ, Greenwood, SC; 2) J.C. Self Research Institute, Greenwood Genetic Center, Greenwood, SC.

Several lines of evidence suggest that a disruption of activity in imprinted genes, especially those in 15q11-q13, may underlie some cases of autism. Duplications of 15q11-q13 are the chromosome abnormality most commonly seen in patients with autism. Until recently, all the 15q11-q13 duplications reported in patients with autism have involved the maternally derived chromosome 15, suggesting that an overdose of an imprinted gene(s) expressed solely from the maternally derived chromosome 15 may be the critical etiological factor in these cases. In addition, mutations in the gene encoding the methyl-DNA binding protein 2 (MECP2) have been shown to be responsible for Rett syndrome, one of the autism spectrum disorders. Differential methylation of CG cytosines is a hallmark of imprinted genes, and mutations in a methyl-DNA binding protein such as MECP2 may disrupt the regulation of activity in imprinted genes.

Southern blot analysis using methylation-sensitive restriction enzymes has been well established as a diagnostic method to determine the methylation status of a targeted CG cytosine in the 5' exon of the SNRPN gene in 15q11-q13. This test is routinely used to confirm or deny clinical diagnoses of Angelman syndrome (AS) or Prader-Willi syndrome (PWS). In addition, the more recently developed methylation-specific PCR assay has been shown to be a specific and highly sensitive technique to determine the methylation status of targeted cytosines, and is gaining popularity as a method for confirming diagnosis of AS and PWS.

Given the possibility that dysfunction of imprinted genes in 15q11-q13 may underlie some cases of autism, we used both these methods to assess methylation status in the 5' exon of the SNRPN gene in patients from the South Carolina Autism Project. The assessment has been completed for 150 patients at the present time, and no abnormalities in methylation status at this CG cytosine have been observed. Further experiments will focus on the two NotI sites in the 5' end of the UBE3A gene in 15q11-q13.

Association of Mitochondrial Deletions with Glasgow Coma Score in Traumatic Brain Injured Patients. *Y.P. Conley¹, D.W. Marion², M.E. Kerr³*. 1) Health Promotion & Development, Univ Pittsburgh, Pittsburgh, PA; 2) Neurological Surgery, Univ Pittsburgh Medical Center; 3) Acute & Tertiary Care, Univ Pittsburgh, Pittsburgh, PA.

The role of genetic factors in cellular repair following injury is increasingly recognized. Mitochondria are involved with cellular respiration, oxidative phosphorylation, apoptosis and energy production and are genetically programmed to perform these functions, which points to their potential importance in individual recovery following neurologic damage. Independent variables that have been implicated in functional recovery following neurotrauma include the age of the patient and the Glasgow Coma Score (GCS) upon hospital admittance, with increased age and lower GCS associated with poorer prognosis. We investigated a common mitochondrial deletion, del7436, in a cohort of 49 patients admitted with severe traumatic brain injury (GCS = 8) at the University of Pittsburgh Medical Center within the Brain Trauma Research Center. The mitochondrial DNA (mtDNA) was extracted from cerebrospinal fluid taken 12 hours post injury. Initially a pair of primers outside of the deletion region were utilized to make sure mtDNA was available in the sample and make sure it was amenable to PCR amplification. All 49 samples amplified using this primer set. A second set of primers was used to determine the presence or absence of the del7436 polymorphism. We found that the frequency of the del7436 deletion was much higher than expected (81.6%) and the presence of the deletion was associated with a lower GCS score ($p = .02$) after controlling for age. The presence of the deletion occurred significantly more often in those with a GCS of 3-6 versus 7-8. Also of interest is the fact that 5 of these 49 samples showed heteroplasmy for this region of the mitochondrial genome in addition to housing a deletion. This study demonstrates that mtDNA status following neurotrauma may play a role in the acute presentation and prognosis of brain injured patients and suggests that further investigations are warranted.

Mitochondrial DNA mutations in non-melanoma skin cancer: Possible genetic selection in tumorigenesis. *W. Giraldo-Rosa, A.C. Musiek, R.A. Vleugels, J.E. Sligh.* Division of Dermatology, Vanderbilt Univ Medical Center, Nashville, TN.

Recent publications have found a high frequency of mitochondrial DNA (mtDNA) mutations in a variety of malignancies. These mutations often were present in all of the mtDNA copies (homoplasmic) within the tumors. Proposed theories to explain the role of these mtDNA changes in tumorigenesis have included possible selective growth advantage or random stochastic segregation. We explored the role of mitochondrial DNA (mtDNA) changes in non-melanoma skin cancer (NMSC) using a genetic approach. Paired tissue specimens of NMSC tumors and adjacent tumor-free margin skin were obtained from surgical excisions in our Dermatology clinic. DNA was isolated from the specimens and long extension polymerase chain reaction (PCR) was used to amplify almost the entire mtDNA in a single reaction. The products were visualized using field inversion gel electrophoresis. mtDNA deletions were often the only forms detected in this assay from the margin skin from elderly patients. However, full length mtDNA was detected in the tumors in all cases despite a probable bias of this protocol to selectively amplify deleted mtDNA species. The increased detection of these full length products from tumors that develop within a background of skin harboring extensive mtDNA deletions suggests the possibility of a genetic selection for specific mtDNAs in the process of tumorigenesis. To investigate this possibility further, we analyzed the full length mtDNAs from the tumors at the single base level for point mutations by DNA sequencing of entire mitochondrial genomes. Homoplasmic point mutations in the mtDNA have been detected in our preliminary sequencing data including protein synthesis mutations affecting the mtDNA-encoded rRNA subunits and mutations affecting specific subunits of NADH dehydrogenase (complex I) and cytochrome c oxidase (complex IV). In addition, homoplasmic tRNA mutations have been detected in NMSC cell lines. We propose that a relative lack of mtDNA deletions within NMSC tumor specimens and the finding of a variety of point mutation types in NMSC tumors and cell lines argue against the random segregation theory of mtDNA mutation homoplasmy.

Identification of Novel Mitochondrial Mutations in Dupuytren's Disease Using Enhanced Multiplex DHPLC. A.

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Dupuytren's disease or contracture (DD) is a familial fibroproliferative disorder of late onset affecting the hands. It is extremely common in individuals of Northern European extraction. Genetic studies have yet to identify the genes involved in DD formation. Oxidative stress and production of free radicals maybe important factors in the pathogenesis of DD. Mitochondrial genes are also included in the regulation of apoptosis. DD tissue contains large numbers of myofibroblasts, which dropped by apoptosis during normal wound healing. High numbers of mitochondria have been observed in fibroblasts derived from DD tissue. we investigated the presence of mutations within the mitochondrial genome in twenty Caucasian Dupuytren's disease (DD) cases with maternally transmitted inheritance pattern and twenty controls matched for age, sex and race using an enhanced multiplex Denaturing High Performance Liquid Chromatography (DHPLC) approach. The modified DHPLC involved amplifying the mitochondrial genome in 18 fragments ranging in size from 300-2000bp using a novel proof reading polymerase (OptimaseTM Transgenomic Ltd, UK) with a low misincorporation rate. Fourteen of these fragments underwent subsequent restriction digestion using a combination of 5 restriction enzymes to enable multiplex DHPLC analysis; the remaining four underwent conventional DHPLC. As part of this whole mitochondrial genome-screening approach we confirmed a number of previously reported mutations and additionally identified a large number of novel mutations. Hitherto, unknown heteroplasmic mutation located within the mitochondrial 16s rRNA region was evident in 90 % of cases and absent from all controls. This mutation may be important in the pathogenesis of DD.

Enhanced detection of deleterious mutations by TTGE analysis of mother's and child's DNA side by side. *H. Kwon, D.J. Tan, R. Bai, L.J. Wong.* Molecular and human genetics, Georgetown University Medical Center, Washington, DC.

Mitochondrial DNA (mtDNA) disorders represent a group of heterogeneous diseases that are caused by mutations in mtDNA. A report demonstrated that analysis of 12 most common point mutations and deletions detected only about 6 % of disease causing mtDNA mutations in 2000 patients suspected of mitochondrial disorders, suggesting that a majority of mutations were not identified. Here, we have examined 45 pairs of mother and the affected child, by screening the entire mitochondrial genome with Temporal Temperature Gradient Gel (TTGE), using 32 pairs of overlapping primers. TTGE is an effective mutation detection method. It detects and distinguishes heteroplasmic mutations from homoplasmic mutations. By running mothers and child's DNA samples side by side and sequence only the DNA fragments showing different TTGE patterns, can avoid the excessive sequencing, particularly the benign polymorphisms. Mutations identified by sequencing were further confirmed by PCR/ASO (allele specific oligonucleotide) dot blot analysis or PCR/RFLP. A total 29 differences in sequence between the mother and child pair were identified. They are A189G, A235G, 249 delA, 303-309 insC, 311-315 insC, T414G, C497T, A1438G, C1428T, A3397G, T5561C, T5580C, G5821A, C5840T, A8326G, T10591G, G12207A, A12333G, C12239T, G12372A, G15995A, T16184-16192insCC, T16189C, C16223T, C16278T, C16294T, A16309G, G16390A and T16519C. Among these mutations, 11 of them are novel. The most significant are the A8326G and G15995A mutations, both were found in an affected female. A8326G is located at the anticodon region, right next to the first nucleotide of the triplet codon and it is invariable throughout evolution from yeast to human. G15995A mutation occurs at a stem region that results in the disruption of the first base pair of the stem region at the anticodon loop of tRNA pro and is highly conserved throughout evolution from sea urchins to mammals. Although many mutations were found in the non-coding regions, they may affect RNA transcription, RNA processing sites, and protein translation. Further functional studies will be necessary to determine the significance of these mutations.

Leigh syndrome associated with a homoplasmic A8344G MERRF mutation. *T.W. Prior¹, G.E. Herman², C.Y. Tsao³, D.R. Boué⁴, J.F. Atkin².* 1) Pathology, Ohio State Univ, Columbus, OH; 2) Pediatrics,; 3) Neurology,; 4) Pathology, Children's Hospital, Columbus, OH.

Leigh syndrome, subacute necrotizing encephalomyelopathy, is a progressive degenerative disorder of the central nervous system most often diagnosed in early infancy or childhood. Genetic heterogeneity is well recognized and the etiologies include both nuclear and mitochondrial gene mutations. We describe an infant with a clinical phenotype consistent with Leigh syndrome: hypotonic weakness, motor and mental regression, progressive respiratory insufficiency, abnormal neuroradiological features and death at the age of 23 months. Mitochondrial DNA studies revealed that the infant was homozygous for the common myoclonic epilepsy and ragged-red fibers (MERRF) A8344G point mutation, however ragged-red fibers, myoclonus, myoclonic epilepsy were all absent and respiratory chain enzyme activities were normal. Furthermore, although all maternal relatives tested were heteroplasmic for the A8344G mutation, migraine headaches were the only clinical feature observed consistent with MERRF. This case clearly demonstrates that the symptoms associated with the A8344G mutation are highly variable and the genetic background, secondary gene mutations, environmental factors and the degree of heteroplasmy may modify the phenotype. Lastly, patients with a clinical presentation and neuroradiological features consistent with Leigh syndrome should be screened for the MERRF A8344G mutation in order to provide an accurate diagnosis and genetic counseling.

Mitochondrial DNA mutations in type 2 diabetic patients from Qinghai, China. *Y.-G. Yao¹, Q.-P. Kong¹, P.-L. Geng², Y.-P. Zhang¹*. 1) Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan, 650223 China; 2) Qinghai Medical College, Xining, Qinghai, 810001 China.

Type 2 diabetes mellitus is a heterogeneous disease that characterized by impaired glucose homeostasis. Several studies have suggested that mitochondrial DNA (mtDNA) mutations may play a role in the development of maternally inherited diabetes mellitus and deafness (MIDD). Among those mutations, the A3243G mutation in tRNA-Leu (UUR) was the most prevalent. In the present study, we screened for this mutation by using PCR-restriction fragment length polymorphism analysis (ApaI) in 78 type 2 diabetic patients and 49 normal controls from Qinghai Province, China. In addition, the hypervariable segment I sequences (HVSI) of mtDNA control region were sequenced in all the subjects and were assigned to mtDNA haplogroups according to the Chinese mtDNA phylogeny. Then, Fisher exact tests were performed to test whether the frequency of a specific haplogroup was different between the patients and controls. None was found to harbor the A3243G mutation in total 127 subjects. Nearly all haplogroups (A, N9a, Y, M7b, M7c, M8a, C, Z, M9a, B4, B5, D5, D, F) identified in type 2 diabetic patients were present in the normal controls. Haplogroups A, B, and D showed higher frequency in the normal controls (A: 10.2%; B: 20.4%; D: 34.7%) than in the patients (A: 5.1%; B: 12.8%; D: 23.1%), but the overall haplogroup distributions were not statistically significant different ($P > 0.05$) between the patients and the controls. Our results were in generally agreement with previous suggestion that mtDNA mutation was not a major cause for type 2 diabetes mellitus in Chinese, but still leaves room for further study with large sample size.

REAL TIME PCR TECHNIQUE ON MITOCHONDRIAL DNA STUDIES. *B. Rodríguez-Santiago¹, S. López², O. Miró², J. Casademont², V. Nunes¹.* 1) Centre de Genètica Mèdica i Molecular-IRO, Hospitalet de Llobregat, Barcelona, Spain; 2) Grup de Recerca Neuromuscular, Hospital Clínic, IDIBAPS, Barcelona, Spain.

A rapid and sensitive method has been developed in our laboratory to study **the relative abundance of mitochondrial DNA (mtDNA)** in different diseases with mitochondrial involvement. **Real time PCR** for mitochondrial **ND2 (mtND2)** and nuclear **r18S** sequences were performed in a fluorescence temperature cycler **Lightcycler** using the **SYBR Green I** dye. A known amount of DNA was serially diluted and amplified for the mtND2 and the r18S sequences to obtain two standard log-linear regression lines. The DNA samples with an unknown mtDNA amount are amplified following the same standard conditions by continuously monitored PCR to obtain the threshold cycle number (Ct). The Ct value of mtND2 and r18S amplification is interpolated in the standard log-linear regression lines to obtain the **mtND2/r18S ratio** used to compare patients and controls. Following this strategy our group has studied the abundance of mtDNA in **Alzheimers disease (AD)** and, recently, the effect on mtDNA caused by different treatments in **HIV** infected patients. In **AD**, DNA samples from three **brain** areas (cerebellum, hippocampus and frontal cortex) of 12 AD patients and 7 controls, and from blood of 17 patients and 27 controls were analysed. Frontal cortex from **AD** patients showed a 28% reduction in their mtDNA contents. In **HIV**, DNA samples of **lymphocytes** from five groups of infected patients were studied. One group included only patients who had not received treatment for the infection. The other groups were treated with different drug schedules for at least 6 months. All groups of treated patients were compared with the naive group. We found **significant mtDNA reduction** in the groups receiving **indinavir (d4T)** and **didanosine (ddI)**. The quantification by **SYBR Green I** with the **LightCycler** also offers interesting perspectives to analyse the **relative expression** of several mitochondrial mRNAs. (Supported by grants *Fundació La Marató TV3 2002/97* and *FIPSE 3102/00*).

Real time quantitative PCR analysis of mitochondrial DNA in patients with mitochondrial disease. *L.C. Wong, R.K. Bai.* Inst for Mol & Human Gen, Georgetown Univ Medical Ctr, Washington, DC.

Molecular diagnosis of mitochondrial DNA disorders is usually focused on point mutations and large deletions. In the absence of detectable mtDNA mutations, abnormal amount of mtDNA, either depletion or elevation, can be indicative of mitochondrial disorders. The amount of mitochondrial DNA (mtDNA), however, varies among individuals of different age and among different tissues within the same individual. In order to establish a range of mtDNA levels, we have analyzed 350 muscle and blood specimens from patients suspected of mitochondrial disorders using real time quantitative PCR method. The copy numbers of mtDNA and 18S rRNA gene were calculated from the standard curve using Taqman probes; 6FAM-5TTACCGGGCTCTGCCATCT3-TAMRA and VIC-5AGCAATAACAGGTCTGTGATG3-TAMRA for mtDNA and 18S rRNA gene (nuclear DNA, nDNA) respectively. The copy number ratio of mtDNA to nDNA was used as a measure of mtDNA content in each specimen. The mtDNA content in muscle increases steadily from birth to about 5 years of age, after that it stays at about the same level. On the contrary, the mtDNA content in blood decreases with age. The amount of mtDNA in skeletal muscle is about 5 to 20 times higher than that in blood. About 7% of patients had mtDNA levels in muscle below 20% of the mean of age-matched group and about 10% of patients had muscle mtDNA levels 2 to 16 fold higher than the mean of age-matched group. Both groups of patients with mtDNA depletion and proliferation had significant clinical manifestation of mitochondrial disease in addition to abnormal respiratory enzymes and mitochondrial cytopathies. Cardiomyopathy, lactic acidosis, abnormal brain MRI findings, hypotonia, developmental delay, seizure, and failure to thrive are general clinical pictures of the patients with mtDNA depletion. The average age of this group of patients is 4.2 years. Mutations in nuclear genes such as thymidine phosphorylase, DNA polymerase gamma, and thymidine kinase are probably the cause of mtDNA depletion. Our results demonstrate that real time quantitative PCR is a valuable tool for molecular screening of mitochondrial disease.

Mitochondrial effect on risk of developing late-onset Alzheimer disease (AD) is modified by gender. *M.A. Pericak-Vance¹, J.M. van der Walt¹, K.K. Nicodemus¹, E.R. Martin¹, C.C. Kroner¹, W.K. Scott¹, D.E. Schmechel¹, G.W. Small², P.M. Conneally³, A.M. Saunders¹, J.L. Haines⁴, J.R. Gilbert¹, J.M. Vance¹.* 1) Duke Univ; 2) Univ of California, LA; 3) Indiana Univ; 4) Vanderbilt Univ.

We examined the association of mitochondrial (mt) DNA sequence variation with AD in a Caucasian sample of 467 cases and 353 controls using logistic regression to test the effect of mt and SNPs. Gender and APOE were used as covariates. However, adjusting for APOE in our data did not significantly change results and thus was not included in subsequent analyses. Previous studies had suggested haplogroups (hpg) K and U were protective against AD but only when APOE was included. SNP combinations were used to assign individuals to a pre-defined European hpg (H, I, J, K, T, U, V, W, X). K hpg individuals showed a decrease in risk (OR=0.57; 95% CI, 0.34-0.97; p= 0.04) of AD versus those with the common H hpg confirming a previously reported association. The 10398G allele was significantly protective (OR=0.70; 95% CI=0.51-0.95; p=0.02). Stratification by gender was significant demonstrating striking differences in the levels of association when the sexes were considered separately. Males classified as hpg U showed an increase in risk (95% CI, 1.16-5.37; p=0.02) of AD versus the most common hpg H. This result is compelling as U is also a risk factor for Parkinson disease in males (JM van der Walt et al., ASHG 2002). Females did not show a significant effect with the U haplogroup (OR=0.65; 95% CI=0.37-1.15; p=0.14) and in fact the association trended in the opposite direction. The male specific results do not support previous reports that found U protective. The risk of AD was decreased in males who carried the 10398G allele (OR= 0.53; 95% CI, 0.32-0.89; p=0.02) versus males that carried the A allele. There was no significant effect in females for 10398G (OR=0.80, 95%CI, 0.53-1.22) although the OR was < 1.00. In summary we have extended our understanding of the effect of mt sequence variation on AD risk showing that mt is independent of APOE and that gender plays a role in the expression of this risk.

Program Nr: 1941 from 2002 ASHG Annual Meeting

Mitochondrial DNA deletions in skin from patients with non-melanoma skin cancer. *J.E. Sligh, A. Eshaghian, R.A. Vleugels, A. Slater, S. Stokes.* Division of Dermatology, Vanderbilt Univ Medical Ctr, Nashville, TN.

The potential role of mitochondrial DNA (mtDNA) changes in cutaneous photoaging was explored using a genetic approach. Photodamaged tumor-free margin skin was obtained from non-melanoma skin cancer (NMSC) patients undergoing skin cancer excision by Mohs micrographic surgery in our Dermatology clinic. DNA was isolated from the specimens and long extension polymerase chain reaction (PCR) was used to amplify 98 percent of the mitochondrial genome in a single reaction. The PCR products were visualized using field inversion gel electrophoresis. mtDNA deletions were abundant in margin tissue specimens from older patients and were often the only forms detected in this assay. These mtDNA deletions were characterized to investigate their utility as possible biomarkers of cutaneous photoaging. The observed mtDNA deletions were typically flanked by 3 bp to 15 bp direct or indirect repeats. The majority of deletions contained within this photodamaged skin did not match any previously reported mtDNA deletion contained within the Mitomap database of human mtDNA variations (<http://www.gen.emory.edu/mitomap.html>). A novel complex triple deletion was also found in the mtDNA from the skin of one patient. Some mtDNA deletions were detected from the skin of multiple individuals including a novel 6278 bp deletion whose frequency approached that of the previously well characterized 4977 bp common deletion. In conclusion, we have characterized many new mtDNA deletions from aging skin which support the use of mtDNA mutations as biomarkers of photoaging in the skin. Indeed, the mtDNA of the skin appears to be a prime target for deletion mutations associated with aging when compared to other tissues.

Identification of a novel candidate gene in the iron-sulfur pathway implicated in ataxia-susceptibility: human gene encoding Hsc20, a J-type co-chaperone. *G.F. Sun, L.E. Vickery, J.J. Gargus.* Dept Physiology & Biophysics, Univ California, Irvine, Irvine, CA.

Iron-sulfur proteins participate in a wide range of biochemical processes including many that are central to mitochondrial electron transfer and energy metabolism. Friedreich ataxia brought focus to the importance of this class of proteins in human neurological disease since loss-of-function alleles of frataxin produce functional mitochondrial defects in patients, and nulls of the yeast ortholog, *YFH1*, display a respiratory-deficient phenotype and a defect in Fe-S cluster formation. Other hereditary ataxia syndromes, including X-linked sideroblastic anemia with ataxia, seem to share these features. Recently, several genes encoding proteins involved in the biogenesis of Fe-S proteins have been identified in bacteria and yeast, and their human homologs are functional candidate genes in these syndromes. One of these genes, *hscB* in bacteria (*JAC1* in yeast) encodes a J-type co-chaperone designated Hsc20 that regulates interactions between the Fe-S cluster assembly protein IscU and the hsp-70-class molecular chaperone HscA/Hsc66. To set the stage for an analysis of the potential role of this gene in human disease, we defined the human *HscB* cDNA, its genomic locus and its pattern of expression in normal human tissues. A search of the human EST database with the *E. coli* HscB protein sequence revealed several human EST homologs, and the complete cDNA sequence of the human *HscB* was defined by sequencing an EST clone. The human *HscB* cDNA encodes 235 amino acids, including a putative mitochondrial import leader. Two BAC clones previously mapped to chromosome 22q11-12 contained most of the genomic sequence. The *HscB* gene is composed of six exons and five introns. Northern blot analyses of RNA from adult and fetal tissues defined a pattern of expression in mitochondria-rich tissues. A 1.4 kb transcript is expressed moderately in adult striate muscle, and weakly in adult kidney and liver. A 2.8 kb transcript is abundantly expressed in both adult and fetal liver. The expression pattern of *HscB* is compatible with its implied role in mitochondrial energetics and related disease phenotypes.

Program Nr: 1943 from 2002 ASHG Annual Meeting

Analysis of the neurotoxicity of the amyloid Ab peptides in *Drosophila melanogaster*. *O. Blard*¹, *A. Laquerriere*², *D. Champion*¹, *T. Frebourg*¹, *M. Lecourtois*¹. 1) INSERM EMI 9906, Faculty of Medicine, 76183 Rouen, France; 2) Department of Pathology, CHU de Rouen, 76031 Rouen, France.

Alzheimer's disease, the first cause of neurodegenerative dementia, is characterized by two cerebral lesions, senile plaques resulting from the deposition of Ab peptides and intracellularly neurofibrillary tangles which the main component is the microtubule-associated protein tau. If the abnormal production and/or aggregation of Ab peptides clearly represents the key event of the disease, the mechanisms underlying the neurotoxicity of Ab peptides remain controversial. We have used *Drosophila* as an experimental organism to analyze the mechanisms underlying the amyloid toxicity *in vivo* and to identify molecules able to interfere with this pathogenic process. First, we have generated DNA constructs allowing the expression of the Ab peptides in the cytoplasm, in the extracellular space or in the endoplasmic reticulum (ER). Transgenic flies have been then engineered using the Gal4-UAS system to express the human amyloid peptides both in the photoreceptor neurons and other cells. Analysis of the biological effects associated with the expression of these different Ab peptides will be presented.

Human genetic response to *Bacillus anthracis* spores. M.R. Altherr¹, S. Wachocki¹, T. Brettin¹, R. Gottardo¹, R. Iyer¹, R.A. Robison³, J.M. Ettinger². 1) B and; 2) NIS Divisions, Los Alamos National Laboratory, Los Alamos, NM; 3) Dept.Micro.,BYU, Provo, UT.

The bacterium *Bacillus anthracis* has been the focus of investigations for over one hundred years. Current events have renewed interest in this organism and its pathological consequences. Recent studies have demonstrated that spores germinate within the phagolysosome. However, the genetic response of the host immediately following the uptake of spores remains obscure. In this study, we have used established cell models exposed to spores from the attenuated *B. anthracis* Sterne strain. While the Sterne strain lacks the pXO2 plasmid and the genes encoding virulence factors associated with capsule formation, it has all the genetic information required for spore germination and release from the host cell. Therefore, the use of Sterne spores should not compromise these findings. We characterized the transcriptional response to *B. anthracis* spores using Affymetrix U133A Gene Chips at four time points using four completely independent replicates. Of the approximately 22,000 genes represented on the U133A array, 2,032 probes pairs exhibited a statistically significant response to the spore challenge in either or both cell lines at one or more time points when compared to an unchallenged control. Cells challenged with latex beads behaved like the unchallenged control and were quite distinct in their transcriptional response when compared to cells challenged with spores. Interestingly, there was significant heterogeneity in the response of two widely used model cell lines. Nevertheless, a total of 254 genes were common responders in both cell lines. Of these, 192 have been localized and all chromosomes are represented. 122 have some annotation in terms of ontology descriptions suggesting a role in a defined biological process, cellular component or molecular function. This means that 50 percent; of the responding genes have no suspected function. 75 percent; of those with suggested roles can be organized into one of seven functional groups. Of these, 33 contain putative polymorphisms in their coding sequence.

Program Nr: 1945 from 2002 ASHG Annual Meeting

Absence of MECP2 Mutations In Patients From The South Carolina Autism Project. *R.C. Michaelis¹, F. Lobo-Menendez¹, K. Sossey-Alaoui¹, S. Copeland-Yates¹, J.M. Bell², S.M. Plank², S.O. Sanford¹, C. Skinner¹, R.J. Simensen¹, R.J. Schroer¹.* 1) JC Self Research Inst, Greenwood Genetic Ctr, Greenwood, SC; 2) Presbyterian College, Clinton, SC.

The methyl-CpG binding protein 2 (MeCP2) gene has recently been identified as the gene responsible for Rett syndrome, one of the autism spectrum disorders. Most female patients with MeCP2 mutations exhibit the classical features of Rett syndrome (RS), including autistic behaviors. Most male patients with MeCP2 mutations exhibit moderate to severe developmental delay/mental retardation. Ninety nine patients from the South Carolina Autism Project (SCAP) were screened for MeCP2 mutations, including all 41 female patients from whom DNA samples were available plus the 58 male patients with the lowest scores on standard IQ tests and/or the Vineland Adaptive Behavior Scale. No pathogenic mutations were observed in these patients. One patient had the C582T variant, previously reported in the unaffected father of an RS patient. Two other patients had novel single nucleotide polymorphisms in the 3' UTR of the gene, G1470A and C1516G. These variants were seen in 12/82 and 1/178 phenotypically normal male controls, respectively. The findings from this and other studies suggest that mutations in the coding sequence of the MeCP2 gene are not a significant etiological factor in autism.

The myotonic dystrophy type 2 (DM2) CCTG expansion does not reduce *ZNF9* transcript levels or prevent splicing. *L.P.W. Ranum, M.L. Moseley, D.D. Jin, C.L. Liquori, J.W. Day.* Inst of Hum Genet, Univ of MN, Minneapolis.

We recently demonstrated that DM2 is caused by a CCTG expansion in intron 1 of the *zinc finger protein 9 (ZNF9)* gene, supporting a gain of function RNA model of DM pathogenesis. The DM2 expansion is huge, with CCTGs that can exceed 44 kb (mean ~20 kb). Similar to DM1, RNA containing the repeat accumulates as nuclear foci. Because repeat expansions decrease transcription in loss of function disorders (FA, FMR) and haploinsufficiency has been suggested to play a role in DM1, we performed a series of experiments to determine if the DM2 expansion interferes with normal *ZNF9* transcription and to define what portion of the *ZNF9* transcript is present in the nuclear foci. Consistent with normal splicing, RNA in situ hybridization in affected muscle and fibroblasts indicate that transcripts containing exon 1 (5' of the expansion) or exon 5 (3' of the expansion) localize to the cytoplasm but not to the CCUG-containing nuclear foci. Probes to sites distributed throughout intron 1 detect transcripts as small punctate signals that are evenly dispersed throughout the nucleus, but that do not co-localize to the much larger nuclear CCUG containing foci. The effects of the CCTG expansion on *ZNF9* expression were independently evaluated using monoallelic mouse-human cell lines containing either a normal or an expanded (~1000 repeats) human DM2 allele. RT-PCR between exons 2 and 1 amplified spliced human transcripts from cells containing either the normal or the expanded allele, and Northern analysis showed comparable levels of human *ZNF9* mRNA with no evidence of unprocessed transcripts. Similarly, equal transcript levels were detected by Northern analysis in diploid fibroblasts from either normal or affected individuals. Taken together, these results indicate that the DM2 expansion does not prevent transcription or splicing of *ZNF9*, the repeat-containing nuclear foci do not contain intron 1 or other portions of the transcript, and the DM2 expansion does not reduce allele specific or overall *ZNF9* mRNA levels. Our results are consistent with a gain rather than a loss of function model in which CCUG expansions themselves alter cellular function.

Genetic and molecular study of migraine. *M.J. Castro*¹, *I. Alonso*^{1,2}, *P. Maciel*¹, *A. Sousa*^{1,2}, *L. Guimarães*^{1,2}, *C. Fraga*³, *J. Barros*³, *J. Sequeiros*^{1,2}, *J. Pereira-Monteiro*³, *I. Silveira*¹. 1) UnIGENe, IBMC, Porto, Portugal; 2) ICBAS, Porto, Portugal; 3) Hospital de Santo António, Porto, Portugal.

Migraine is a highly prevalent primary headache disorder. The most common form of this disease is migraine without aura (MO), characterized by International Headache Society (IHS) guidelines as unilateral pulsating pain of moderate to severe intensity, aggravated by physical activity. Migraine with aura (MA) shares the same headache symptoms, but is preceded by aura, which are attacks of focal neurological symptoms. A rare autosomal dominant sub-type of MA is familial hemiplegic migraine (FHM) with severe symptoms during the attacks, such as hemiparesis and coma. A significant number of families with FHM have shown genetic linkage to chromosome 19p13; linkage to chromosome 1q has also been detected in some FHM families; however, several FHM families have not presented linkage to any of these *loci*. Mutations in a gene located on chromosome 19p13, the *CACNA1A* gene, that encodes for an α_{1A} -subunit of a brain P/Q-type calcium channel, have been described for different disorders: FHM, spinocerebellar ataxia type 6 (SCA6), and episodic ataxia type 2 (EA-2). To identify new genes and mutations involved in migraine, we ascertained 90 families with MO, MA or FHM and 1) performed linkage analysis in informative families, and 2) screened patients for mutations in the *CACNA1A* gene. Sixty-five families had migraine dominantly inherited, including four with FHM; 14 were isolated cases of migraine, whereas the remaining families had an unknown pattern of inheritance. Linkage analysis for chromosome 19p13 was performed with *CACNA1A* intragenic markers (CAG)_n and D19S1150. Mutation analysis was performed by SSCP and sequencing. Mutations already described in the *CACNA1A* gene were not found in our group of families. One family with FHM was excluded to the *CACNA1A* gene by linkage analysis. Three new polymorphisms in exons 13 (C2054G), 20 (T3755A) and 29 (T4898C) were detected. The study proceeds with the screening of the entire coding region of the *CACNA1A* gene and with linkage analysis for the additional *loci* implicated in migraine.

Association of Parkin with late-onset Parkinson disease. *S.A. Oliveira¹, W.K. Scott^{1,2}, E.R. Martin¹, J.M. Stajich¹, L.T. Middleton², J.L. Haines², M.P. Winn¹, M.A. Pericak-Vance^{1,2}, J.M. Vance^{1,2}, GSK Collaborative Parkinson Disease Group².* 1) Center for Human Genetics (CHG), PO Box 2903, Duke University, Durham, NC; 2) Duke CHG/ GlaxoSmithKline PD Genetics Collaboration.

Mutations in Parkin, an E2-dependent ubiquitin protein ligase, cause autosomal recessive juvenile parkinsonism, but its role in the late-onset form of PD is not firmly established. In a genomic screen of PD we detected linkage to D6S305, lying in the Parkin gene (LOD=5.47), in families with at least one member with an age at onset (AAO) < 40 years (early-onset PD families). Parkin mutations were identified using denaturing HPLC and sequencing. Genotyping was performed using the oligonucleotide ligation assay. Three intronic and three exonic single nucleotide polymorphisms (SNP) were used for association studies in families not known to carry Parkin mutations. 607 PD patients (average AAO \pm SD = 61.8 \pm 13.5 y), 872 unaffected controls (average age at examination \pm SD = 67.5 \pm 12.3 y), and 101 individuals with unclear diagnosis were included from a total of 397 families. Mutations were identified in 18% of early-onset families and 2% of late-onset families, which together comprise 4% of the total number of families. The AAO of patients with Parkin mutations ranges from 12 to 71 years. The most common change was a deletion of 40 bp in exon 3. Excluding exon 7, average AAO for patients with Parkin mutations was 31.5 years. Three mutations in exon 7 (Arg256Cys, Arg275Trp and Asp280Asn) were observed in individuals with late AAO (average AAO = 51 years), mostly in the heterozygous state. Combining our data set and that of the literature, these mutations were not found in over 900 unrelated control chromosomes. We detected no significant association ($p > 0.05$) of any of these SNPs with PD. These findings indicate that Parkin mutations do contribute to the common, late-onset form of PD. Importantly, it appears that Parkin mutations in the heterozygous state may act as susceptibility alleles for late-onset PD, especially those in the first ring finger (exon 7).

Novel point mutations in two compound heterozygous cases of Friedreich's Ataxia. *J.R. Leib¹, C. Stolle², J.P. Taylor¹, J.B. Caress³*. 1) Neurogenetics Branch, National Institute of Neurological Diseases and Stroke, National Institutes of Health, Bethesda, MD; 2) Genetic Diagnostic Laboratory, University of Pennsylvania School of Medicine, Philadelphia, PA; 3) Department of Neurology, Wake Forest University School of Medicine, Winston-Salem, NC.

Friedreich's ataxia (FRDA) is an autosomal recessive, multisystem degenerative disorder affecting approximately 1 in 40,000 individuals in the United States. FRDA results from loss-of-function mutations involving the X25 gene encoding the protein frataxin at chromosome 9q13. Approximately 96% of Friedreich's Ataxia (FRDA) patients harbor a homozygous expansion of a trinucleotide repeat (GAA) within intron 1 of the X25 gene. In the remaining FRDA cases, the patients are heterozygous at the X25 locus with one typical expanded allele and a point mutation. We report two such compound heterozygous patients with typical, severe FRDA phenotypes. Our first patient is a 25-year-old male with approximately 725 GAA and 7 GAA repeat sequences in intron 1 of the X25 gene. Sequencing revealed that his non-expanded allele also contains a novel point mutation (11-12 del TC) in exon 1 introducing a shift of the reading frame, creating 26 novel amino acids and a premature stop codon at amino acid position 91. Our second patient is a 31-year-old female with approximately 700 GAA repeat sequences in intron 1 and a non-expanded allele with a novel 2 base pair deletion in exon 2 (214-215 del AG). This frame shift mutation introduces 21 new amino acids and a premature stop codon at amino acid position 91. Similar to the expansion, these novel null mutations are predicted to preclude expression of frataxin mRNA.

DISCORDANT PHENOTYPES IN FIRST COUSINS WITH UBE3A MUTATION. *G.A. Molfetta^{1,2}, M.V.R. Munoz³, W.A. Silva-Jr⁴, J. Wagstaff², J.M. Pina-Neto¹.* 1) Dept Genetics, Ribeirao Preto - USP, Sao Paulo, Brazil; 2) Department of Pediatrics, University of Virginia School of Medicine, USA; 3) Clinica Materno Fetal, Florianopolis, SC, Brazil; 4) Center for Cell Therapy and Regional Blood Center, Ribeirao Preto, Brazil.

Angelman syndrome is a neurobehavioral disorder caused by defects of the imprinted UBE3A gene in chromosome 15q11-13. Different genetic mechanisms can result in loss of a functional maternal allele of UBE3A in AS: 70% are maternal 15q11-13 deletions; 7-9% from imprinting defects; 3-5% from UPD; and 4-8% from mutations in the UBE3A gene. The remaining 10-15% have no deletion, imprinting defect, UPD, or UBE3A mutation. UBE3A mutations are more frequent in familial than in sporadic patients and the mutations described so far seem to cause similar phenotypes in the familial affected cases. We describe two affected first cousins who have inherited the same UBE3A mutation from their asymptomatic mothers but presenting discordant phenotypes. The proband shows typical AS features. Her affected cousin shows a more severe phenotype, with asymmetric spasticity that led originally to a diagnosis of cerebral palsy; brain MRI shows schizencephaly associated with cortical dysplasia and subtle white matter atrophy in posterior cerebral areas. We have three hypotheses to this phenotypic discordance: the second cousin has an additional problem (genetic or environmental) besides the UBE3A mutation; the UBE3A mutation is interacting with a genetic variant in the second cousin to cause the severe phenotype; or this UBE3A mutation alone can cause either typical AS or the severe clinical picture seen in the second cousin. This family demonstrates that the presence of a brain malformation does not exclude the diagnosis of AS. Another unusual observation regarding this UBE3A mutation is the fact that it was transmitted from the cousins grandfather to only two sisters among eleven full siblings. As the expected rate of normal carriers within the sibship would be 50% if the maternal grandfather was a normal carrier, we hypothesize that the transmitting grandfather, now deceased, may have been mosaic for this mutation. Financial Support: FAEPA.

Comprehensive Mutation Scanning of RAR/RXR and Vitamin D Receptor genes in Patients with Schizophrenia and other Psychiatric Diseases: Three missense mutations at highly conserved residues. *J. Yan¹, J. Feng¹, J. Chen¹, N. Craddock², I. Jones², E.H. Cook Jr³, D. Goldman⁴, L.L. Heston⁵, S.S. Sommer¹.* 1) Dept of Molecular Genetics, City of Hope, Duarte, CA; 2) Div of Neuroscience, Univ of Birmingham, Birmingham, UK; 3) Dept of Psychiatry, Univ of Chicago, Chicago, IL; 4) Dept of Psychiatry, NIAA, NIH, Bethesda, MD; 5) Dept of Psychiatry, Univ of Washington, Seattle, WA.

Retinoic acid receptors (RAR), retinoid X receptors (RXR) and the Vitamin D receptor (VDR) are involved in the regulation of brain function. Defects in these receptors may contribute to schizophrenia or other psychiatric diseases. To test this hypothesis, the RAR and RXR genes (RAR α , β , γ & RXR α , β , γ) and the VDR gene were scanned by DOVAM-S (Detection of Virtually All Mutations-SSCP), a robotically enhanced multiplexed scanning method. In total, 196 subjects were scanned in each gene (including 100 patients with schizophrenia and a pilot experiment in patients with bipolar disorder (24), autism (24), ADHD (24), and alcoholism (24)). 3.2 megabases of genomic sequences were scanned in total. Seven VAPSEs (Variants Affecting Protein Structure or Expression) and 24 non-VAPSEs were identified. The data define structural variants that may be relevant to disease. Only the RAR β gene showed some potential relationship to schizophrenia. One VAPSE, N307T in the RAR β gene is at a residue that is highly conserved during evolution; e.g., N is conserved in the five available mammalian species, bird species and fish, hinting that these sequence changes will be of functional significance. A further case-control study showed a trend that the frequency of N307T is higher in schizophrenia than that in ethnically matched controls (4/498 vs 1/509). Three of the missense mutations identified at highly conserved residues are likely to be of functional significance. Ultimately some or all these VAPSEs may be associated with clinical phenotypes.

The dystrophin gene is associated with sporadic dilated cardiomyopathy: implications for clinical care. S.S.

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Dilated cardiomyopathy (DCM) is the major indication for heart transplantation. Approximately 30% of all DCM is thought to be inherited, while 70% is sporadic. Mutations in the dystrophin gene have been associated with the uncommon X-linked form of DCM. We hypothesized that missense mutations and other less severe mutations of the dystrophin gene might predispose to the common form of sporadic DCM. To test this hypothesis, 22 kb of genomic dystrophin DNA was scanned with DOVAM-S (Detection of Virtually All Mutations-SSCP) in each of 22 patients with sporadic DCM, including all 79 coding sequences and splice junctions, as well as six alternative exon 1 dystrophin isoforms (484 kb, total). Three putative new mutations (IVS5+1 G>T, K18N and F3228L) and seven polymorphisms were identified. The splice site mutation IVS5+1 is predicted to cause skipping of exon 5, which is within a region containing an actin binding site. The missense mutations occur at amino acids that display substantial evolutionary conservation. Screening of 236 control individuals failed to identify these 3 mutations. CK-MM (creatine kinase, skeletal muscle) was measured in 17 patients. The three patients with putative mutations had CK-MM level of 11,300, 430 and 270, respectively, while the 14 patients without mutations had values ranging from 20 to 200. The first comprehensive mutation scanning of the exons and splice junctions of the dystrophin gene in patients with sporadic DCM suggests that point mutations are associated with sporadic DCM without clinical evidence of skeletal myopathy. It may be prudent to add CK-MM to the clinical analysis of patients presenting with dilated cardiomyopathy in order to identify candidates at high risk for dystrophin mutations.

High throughput mutation detection in the mitochondrial genome using DHPLC. *J. Walter¹, H. Lamb¹, M.A. Marino²*. 1) Applied Genomics, Transgenomic Inc, Crewe, Cheshire, England; 2) Applied Genomics and Molecular Genetics, Transgenomic, Inc., Gaithersburg, MD.

Denaturing High Performance Liquid Chromatography (DHPLC) and the WAVE® System enables automated mutation detection via heteroduplex analysis. This technique is capable of providing high efficiencies when scanning for unknown mutations with very low false positive levels and no reported bias with regard to mutation type (single base changes or insertion deletion mutations). A further advantage is the ability to detect small proportions of a mutant allele in a wild type population. This high level of sensitivity, less than 1% of mutant in a wild type population, has been previously reported with reference to mutation detection in the mitochondrial genome (Smeets et al). Mutation detection in the mitochondrial genome is relatively easy due to its small size (approx. 16kb) however the heteroplasmic nature of the mitochondrial DNA population can result in the presence of mutations at very low levels which are difficult for most techniques to detect.

We report here significant improvements to the previously published technique to allow it to be used as a highly efficient scanning tool for the detection of unknown mutations. These improvements include redesign of the primer sets enabling streamlining of the procedure and the ability to minimise the number of different restriction enzymes used. Software analysis of the mitochondrial genome to reduce the number of DHPLC method conditions necessary whilst maintaining mutation detection efficiency. The use of a novel proof reading polymerase (Optimase™ polymerase) to reduce the number of misincorporations during the amplification procedure; maximising the sensitivity of the technique. Finally transferring the technique to the WAVE® System 3500HT platform enabling screening of the entire mitochondrial genome in less than 6 hours.

Investigating a possible relation between the amino acid variation N158S of the human heat shock protein Hsp60 and an increased susceptibility to Sudden Infant Death Syndrome (SIDS). *A. Teske¹, T. Rothämel¹, W.J. Kleemann², H.D. Tröger¹.* 1) Inst Legal Medicine, Medical Sch Hannover, Hannover, Germany; 2) Inst Legal Medicine, U Leipzig, Germany.

Hyperthermia (among other factors) combined with a dysfunctional heat stress reaction may potentially be life threatening and lead to SID. The gene of the human heat shock protein Chaperonin 60 (Hsp60) has been considered twice in relation to SIDS. Rahim et al. 1996 reported an RFLP in the hsp60 gene, which was characteristic for the 12 SID cases examined. Bross et al. 2001 found the amino acid variation N158S (asparagine to serine) in a single case of 65 SID cases analyzed. They postulate this occurrence as a possible rare disease allele of the hsp60 gene. Our work aims to examine this variation in relation to increased SIDS susceptibility.

The codon for the amino acid 158 (asparagine) is located in exon 5 of the hsp60 gene (position 8748-8843; GenBank Acc. No. AJ250915). The DNA of the SID cases was isolated from blood samples taken during autopsies at the Institute for Legal Medicine of the Medical School Hannover (Germany). The DNA of the controls was isolated from blood samples of children undergoing paternity testing at the same institute. For 31 SID cases and 20 controls, we sequenced a PCR fragment of the entire exon 5 in both directions (note for reviewers: in the final version we expect to show the results for 100 SID cases and 100 controls).

So far, no amino acid variation N158S was found in either of the SID cases or controls. Furthermore, no other mutations in the complete exon 5 of hsp60 could be discovered.

Based on our current results, a direct relation between the amino acid variation N158S in the gene of human heat shock protein Hsp60 and SIDS cannot be confirmed by our experiments.

Mutation of p53 gene in ocular pterygia. *D. Reisman¹, J. McFadden¹, N. Lin¹, R.G. Best¹, G. Lu^{1,2}*. 1) University of South Carolina, Columbia, SC; 2) Genzyme Genetics, Yonkers, NY.

Pterygium is a web-shaped growth of fibrovascular tissue onto the cornea that is continuous with the conjunctiva. It occurs at highest prevalence and most severely in tropical areas, and to a lesser and milder degree in cooler climates. UV-light exposure has been considered to be risk factor for pterygium. Furthermore, chromosome instability in pterygium suggests a DNA repair deficiency. UV-induced p53 mutations have been found to be associated with skin cancer, and sunlight exposure is the most important epidemiological risk in the development of pterygium, thus we hypothesize that interaction between UV-light exposure and somatic mutations in p53 may lead to uncontrolled cell growth in pterygium. Recently, we investigated genetic changes in a small-scale study including 9 pterygium samples and 10 controls. Chromosome instabilities were found in two out of the 9 cases of pterygium, and monoallelic deletion of p53 was detected in 5 cases by FISH analysis. Analysis of p53 protein and mRNA by RT-PCR in cells derived from pterygium patients revealed no detectable p53 expression while p53 protein was readily detectable in control samples. Analysis of the genomic DNA organization of the p53 gene indicates that the p53 gene from control and pterygium-derived cells has the same overall structure and no major rearrangements appear to be responsible for the loss of p53 expression. DNA sequence analysis in a selected sample was found to have the wild type p53 sequence. This is, to our knowledge, the first detection of p53 monoallelic deletion and chromosome instability in pterygium. We conclude from our findings that in a significant frequency of cases of pterygium, the p53 gene has undergone a monoallelic deletion. The remaining allele appears to remain wild type, and is transcriptionally silenced. The mechanism of silencing the p53 gene is currently under investigation and may include alternations in regulatory regions of the gene or methylation of important regions. (This study was supported by a Grant GA20037 (to G.L.) from Fight for Sight).

Dopamine Transporter Genotype (DAT1) Affects Stimulant Response in Children with ADHD. *M.A. Stein¹, I. Waldman², C. Sarampote¹, K. Seymour¹, A. Robb¹, C. Conlon¹, Z. Yang³, E.H. Cook³.* 1) Psychology, Psychiatry, and Pediatrics, Children's National Medical Ce, WASHINGTON, DC; 2) Psychology, Emory University, Atlanta, Ga; 3) Child and Adolescent Psychiatry, The University of Chicago, Chicago, Il.

Background: Stimulant medications, such as Methylphenidate (MPH), are the most common and effective treatments for ADHD. MPH acts primarily by inhibiting the dopamine transporter (DAT1). DAT1 codes for a protein responsible for the reuptake of dopamine from the synapse back into presynaptic terminals. The 480 bp allele of DAT1 has been associated with ADHD in several within family studies. Objective: We hypothesised that the presence of the 480 bp (i.e. high risk) allele would be associated with a positive response to stimulant medication in children with ADHD. Design/Methods: Children with ADHD (n = 42), ages 5-16 (mean = 8.8), underwent a 4 week, double blind, placebo controlled, crossover trial. Children were genotyped for DAT1 and evaluated on placebo and Concerta MPH at 3 dose levels: 18 mg, 36 mg and 54 mg qd. Parents and clinicians who were blind to genotype and medication status rated ADHD symptoms and stimulant side effects each week. Repeated measures analysis of variance was used to test differences in stimulant response by DAT1 genotype. Results: In contrast to those with no copies of the high risk allele, with increasing Concerta MPH dosage children with 1 or 2 copies were more likely to display a linear decrease in total ADHD symptoms ($R^2 = .19$, $p = .016$). Conclusions: In the presence of one or 2 copies of the DAT1 high risk allele, ADHD symptoms declined linearly with increased Concerta MPH dose. Replication with a larger sample and further study of reasons for lack of efficacy are ongoing.

Chromosome 15 and autism: Clinical characterization of phenotype. *M. Cuccaro¹, S. Donnelly¹, S.A. Ravan², R.K. Abramson², L. Elston¹, K. Decena², H.H. Wright², M.A. Pericak-Vance¹.* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) WS Hall Psychiatric Institute, Univ of South Carolina, Columbia, SC.

Autism (AutD) is a complex neurodevelopmental disorder. Epidemiologic and genetic analyses indicate that AutD is genetically heterogeneous and associated with a very wide range of clinical manifestations. Chromosomal region 15q11-q13 is one candidate region based on cytogenetic linkage and association findings. Ordered subset analysis (OSA) is an analytic method that identifies a homogenous subset of individuals that contributes to linkage in a particular chromosomal region. Using a factor derived from restricted and repetitive behaviors as the covariate in OSA, a subset of 23 AutD families were identified as contributors to linkage on Chr 15 (OSA N=46; 23 sibpairs). We compared these Chr 15 AutD sibpairs with the remaining AutD families that did not show evidence for linkage on composite indices from the Autism Diagnostic Interview-R (ADI-R) and Vineland Adaptive Behavior Scales (VABS). Between group comparisons revealed that the OSA defined subset was more impaired on a measure of social functioning (VABS Socialization $t=2.72$, $p=0.007$). In addition the OSA defined AutD group showed a trend toward significance reflective of lower overall adaptive functioning (Adaptive Behavior Composite $t=1.91$, $p=0.058$). The groups did not differ on either the ADI-R or VABS communication indices. These results are suggestive of a greater degree of social impairment in the OSA defined Chr15 AutD group. The VABS Socialization domain has been proposed as a quantitative index of social impairment in AutD. The results are consistent with the hypothesis that increased repetitive behavior exerts an effect on social and behavioral functioning. Additional efforts to investigate different phenotypic expression in AutD populations for application to genetic analysis will be extremely valuable in delineating the boundaries of AutD.

SELDI-TOF mass spectrometry as a tool for biomarker discovery in autism. *S.J. Walker¹, A.S.L. Xu², K.E. Vrana¹.*
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Autism is debilitating neuropsychiatric condition typically diagnosed between ages 3-6 years. While there is evidence of a genetic component associated with autism spectrum disorder (ASD), an "autism gene" has not yet been identified. Growing evidence suggests, however, that there are hallmark/physiologic abnormalities in autistic children that might be manifested in circulating proteins. Identifying these proteins will provide a way to study the important functional genomic changes associated with ASD. In this pilot study, serum from four autistic individuals and four non-autistic siblings (samples selected and purchased from the Autism Genetic Research Exchange [AGRE]) was assayed. The global differential expression of serum proteins in autistic samples compared to non-autistic controls was detected and analyzed using the surface enhance-enhanced laser desorption ionization time-of-flight (SELDI-TOF) mass spectrometry technology (CIPHERGEN Biosystems, Inc.; Fremont, CA) to profile patterns of lower molecular-weight serum proteins. The eight samples were assayed on a variety of chromatographic ProteinChip® array surfaces, under a variety of conditions designed to capture a subset of proteins, in an effort to identify protein profile differences between affected and non-affected samples. The pilot experiment (in which »750 mass spectra were generated) has so far revealed pattern differences that are apparent in serum fraction #4 (eluted at pH 5 column fractionation) assayed on the weak cationic exchange (WCX2) chip surface. From these data, it appears the autistic samples are lacking (or have a very reduced amount, compared to non-autistic samples) proteins at 6457, 6550, 17,440, and 28,100 daltons. These experiments demonstrate the potential for using the SELDI-TOF MS ProteinChip® technology to illuminate protein expression differences which, in turn, may lead to a better understanding of the functional genomic changes that underlie autism spectrum disorders. These studies were funded by the Wallace Foundation and the Autism Research Institute (ARI).

Application of IR-PCR for physical mapping of the autistic disorder candidate region on chromosome 15q12-q13. *S.-J. Kim, M.M. Menold, J.M. Vance, M.A. Pericak-Vance, J.R. Gilbert.* Department of Medicine and Center for Human Genetics, Duke University Medical Center, Durham, NC.

Chromosome 15q12-q13 has been implicated in the genetic etiology of autistic disorder (AutD). The physical mapping of this region is important to identify AutD candidate gene or genes on chromosome 15q12-q13. Previously, we generated a genomic contig map of the putative peak AutD candidate gene region. To identify 5' transcription sites and AutD candidate genes within the approximately 1.2-1.5 Mb contig, 28 BAC, PAC and P1 clones that span the region and contain Not I and numerous Eag I, BssHII and SacII sites were analyzed using Island Rescue PCR (IR-PCR). Eighty-three unique EagI, BssHII and SacII related sites were cloned and sequenced. We identified 45 unique CpG islands that met full CpG island criteria. The human draft sequence for 15q11-13 is unfinished. We performed a computation analysis of known sequence from the same region for CpG islands and identified 92 predicted CpG islands compared to our experimental IR-PCR results of 83 clones. The IR-PCR CpG islands were mapped onto the human chromosome 15 draft sequence assembly by BLAST analysis. Of the total 83 clones, 64 have been mapped onto the incomplete human genomic draft contig in this region. Of the 45 IR-PCR clones that met full CpG island criteria 31 were mapped to this region. We have used these CPG islands clones, derived from known genomic clones, to verify contig location and order in the assembly of the 15q12-q13 draft sequence. The assembly of 15q12-q13 draft contig was also compared with the comparative mouse map. Using a combination of northern blot analysis of CpG island clones and BLAST sequence analysis we have identified and mapped twenty clones that showed expression on northern hybridization to human fetal brain RNA, or a significant match to a known cDNA or EST.

Association studies of Renin-Angiotensin System (RAS) gene polymorphisms with clinical and histological features in primary IgA nephropathy (Berger's disease). *M. Brugnara¹, B. Brezzi², C. Patuzzo¹, M. Gomez-Lira¹, A. Lupo², G. Maschio², R. Magistroni³, A. Turco¹.* 1) Department of Mother & Child, Section of Genetics, University of Verona School of Medicine, Verona, Italy; 2) Division of Medical Nephrology, Unive of Verona, Italy; 3) Dept of Internal Medicine, Div of Nephrology, Univ of Modena, Italy.

With a population prevalence of up to 1.3%, IgAN represents the most common primary glomerular disease, and a leading cause of end-stage renal disease worldwide. The IgAN phenotype results from the complex interplay of genetic and non genetic factors. The RAS is well known to play a role in promoting kidney damage. In this study we tested whether RAS-gene polymorphisms (pol), already linked to several cardiovascular and kidney diseases, might be associated with clinical and/or histological features of IgAN. 95 unrelated Northern Italian biopsy-proven IgAN patients (pts) (aged 16 to 65y) were analysed for 1) the I/D pol of the angiotensin converting enzyme (ACE) gene, 2) two pol (T174M and M235T) in the angiotensinogen (AGT) gene, and 3) the A1166C pol of the angiotensin II receptor type 1 (ATR1) gene. At the time of biopsy all pts had measurements of their creatinine clearance (mean=98,8 ml/min), proteinuria (mean=1,25 g/die), and blood pressure, expressed as mean arterial pressure (MAP)(mean 100 mmHg). Using a multivariate analysis (ANOVA) we found significant association between the II ACE genotype and raised MAP at the time of biopsy (II pts=108+/-7 mmHg; ID and DD pts=99+/-11 mmHg; p=0,014), the percentage of the glomerular surface involved in sclerotic change (II pts= 28+/-10%-ID and DD pts= 11+/-5%; p=0,0006), and the chronicity index (II pts=5-ID and DD pts=3; p= 0,013). We also found that the AGT TT genotype 235T was associated with elevated proteinuria (TT pts= 1,8 +/-1 g/die-MT and MM pts= 1+/-0,8 g/die; p =0,009), high PAM (TT pts= 106+/-10 mmHg; MT and MM pts= 98+/-11 mmHg; p= 0,015), and the amount of mesangial proliferation (TT pts= 40%- MT and MM pts= 22%; p= 0,014). Thus, we present evidence that in our IgAN population some RAS gene variants appear associated with worsened clinical and histological outcomes.

Mutations in the SPINK 1 gene in idiopathic pancreatitis and neonatal hypertrypsinemia. *M. Gomez-Lira¹, D. Bonamini¹, C. Patuzzo¹, C. Castellani², L. Unis³, BM. Assael², PF. Pignatti¹.* 1) Mother & Child & Genetics, Univ Verona, Italy; 2) Cystic Fibrosis Center, Verona, Italy; 3) Transfusional Center, Hospital of Bussolengo, Italy.

Idiopathic chronic and acute recurrent pancreatitis (IP) and neonatal hypertrypsinemia, are genetically heterogeneous pancreatic alterations that have been associated with mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Mutations in the trypsinogen gene have been described in hereditary pancreatitis. Gain of function mutations of the trypsinogen gene lead to pancreatitis by enhancing activity or by preventing activated trypsin from being inactivated. Similar effects could be due to the loss of trypsin inhibitor function. Mutations in the serine protease inhibitor Kazal Type 1 (SPINK1) have been described in some IP patients. In this study we have analyzed the promoter region and the four exon and exon-intron boundaries of the SPINK1 gene in 32 IP patients, in 50 hypertrypsinemic neonates with normal sweat chloride level, and 50 controls. Mutation N34S (the most common mutation described in IP patients) was screened by genomic amplification followed by restriction analysis. Three IP patients (9.3%) and one control individual (2%) carried the N34S mutation of the SPINK1 gene. The 5'UTR and the four exons and exon-intron boundaries of the gene were screened for other mutations by DGGE analysis and sequencing. No other mutation was observed in IP patients that could be associated with an altered function of the SPINK1 protein. Interestingly, the N34S mutation was present in two patients who carried the 5T variant and one patient who carried the L997F mutation in the CFTR gene, suggesting a possible combined effect of the CFTR and the SPINK1 genes. We found no mutation in neonatal hypertrypsinemia indicating that the SPINK 1 gene is not likely to be associated with this phenotype.

Genetic susceptibility in cervical dystonia: confirmation of a role for the dopamine D5 receptor gene. F.

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Cervical dystonia (CD) is one of the most common form of primary dystonia. The pathogenesis of the disease is still unknown, although some evidence suggests a role for genetic factors. Recently, a polymorphism in the dopamine D5 receptor gene (DRD5) was associated to the disease in a British population, suggesting that DRD5 is a susceptibility gene for CD (Placzek et al.). To confirm these data, we performed a case-control study between the microsatellite (CT/GT/GA)_n at the DRD5 locus and two groups of 104 Italian dystonic patients and 104 healthy controls, matched for sex, age and ethnicity. The mean age of cases and controls was 49.6 14.9 years and 49.5 15.0 years respectively. Female to male ratio was 1.08. The frequency of allele 9 was higher in the group of CD patients compared to the control group (odds ratio = 2.44; 95% confidence intervals: 1.14-5.27; p=0.01), resulting in a double-fold increased risk of developing the disease. On the contrary, allele 3 was more common in control subjects compared with the patients group (odds ratio = 0.56; 95% confidence intervals: 0.30-1.06; p=0.06). Both susceptibility and protective DRD5 alleles in the present study were different to those reported in the British study. This is an interesting finding supporting the association between DRD5 and CD. In fact, the (CT/GT/GA)_n microsatellite is located at 5 outside the DRD5 coding region, making a functional role for this polymorphisms very unlikely. Therefore, we suggest that a functional, still unidentified, variant in DRD5 identical in the two populations, could be in linkage disequilibrium with two different (CT/GT/GA)_n alleles, depending on the different genetic background of the Italian and British populations. These results provide further evidence for an association between DRD5 and cervical dystonia, confirming the involvement of dopamine pathway in the pathogenesis of CD.

Drosophila as a model for discovering candidate genes in ADHD. *V. De Luca*¹, *P. Muglia*^{1,2}, *U. Jain*², *V.S. Basile*¹, *J.L. Kennedy*¹. 1) Neurogenetics, CAMH , Toronto, Ontario, Canada; 2) Adult ADHD Clinic, CAMH, Toronto, Ontario, Canada.

Attention deficit hyperactivity disorder (ADHD) is a prevalent psychiatric condition in children and follow up studies have indicated that 22-33% of patients continue to suffer from ADHD during late adolescence and adulthood. The risk factors for ADHD may be determined by additional mechanisms beyond the dopamine transporter and receptors. We are exploring new methodology for discovering these mechanisms. In drosophila, such an additional factor could be protein kinase G (PKG) that affects food-search behaviour. Here we initiated studies with the human homologue of PKG, the PRKG1 gene. The aim of this study was to investigate for the presence of linkage disequilibrium between the protein kinase G gene (PRKG1) and adult ADHD in a sample of 125 nuclear families. Genotyping data for the C2276T polymorphism were analyzed using the Transmission Disequilibrium Test (TDT). Sixty three nuclear families were informative for the TDT on C2276T polymorphism, which showed no preferential transmission of either allele (chi-square= 0.778, df=1, p= .316). These findings exclude a direct involvement of this genetic marker of the Protein kinase G gene in the pathogenesis of ADHD, but further studies of inattentive versus hyperactive subtypes and other SNPs at this locus, are warranted.

High Throughput Detection of Small Genomic Insertions and Deletions by Pyrosequencing technique. *D. Guo, Y. Qi, R. He, P. Gupta, D. Milewicz.* Dept Internal Medicine, Univ Texas/Houston Med Sch, Houston, TX.

The Human Genome Project has uncovered thousands of DNA sequence variants in the human genome including nucleotide tandem repeats, single nucleotide polymorphisms, and small insertions and deletions. These variants have been widely used as genetic markers for inherited disorder mapping, population genetics studies and forensic DNA analysis. Numerous techniques have been developed for high throughput detection of chromosomal variations such as (SNPs) and nucleotide tandem repeats. However, high throughput methods for detection of insertions and deletions of a small number of nucleotides are limited. In this report, we demonstrate that pyrosequencing technique can be used for a high throughput assay of these variations. Serial nucleotide deletions with 2bp, 5bp, 8bp, and 100bp respectively, were created in exon 8 of the versican gene, and then assayed by the pyrosequencing technique. The results showed that the pyrosequencing technique is able to detect up to 100bp nucleotide deletions and insertions. By modifying sequence program, this technique is able to detect a G deletion in FBLN5 and a complicated combination of an 11 bp nucleotide deletion and a 4 bp insertion in intron 33 of FBN2. Our results indicated that pyrosequencing technique is applicable for high throughput genotyping of nucleotide insertions and deletions in large populations for the study of genetic predisposition of disease.

A rare Eag I polymorphism that may confound fragile X diagnosis. *C.S. Dobkin¹, X.-H. Ding¹, G.E. Houck¹, E. Mitchell², W.T. Brown¹, S.L. Nolin¹.* 1) Dept Human Genetics, NYS Inst Basic Research, Staten Island, NY; 2) Las Vegas Perinatal Associates, Las Vegas, NV.

Fragile X diagnostic Southern analysis can detect deletions and restriction fragment length polymorphisms in addition to the triplet repeat expansion mutation. We have identified a rare Eag I polymorphism in the fragile X (FMR1) gene that reduces the normal EcoR I-Eag I restriction fragment from 2.8 kb to 1.9 kb. The polymorphism was identified in a boy (DOB 1/30/96) who was tested for the fragile X mutation because of delayed speech. This restriction polymorphism had originally been identified as a deletion by another laboratory. Southern analysis indicated that the mutation was located in intron 1 and that it was present on one of the mother's alleles. Sequence analysis showed that this polymorphism is a G-C transversion that results in a new Eag I site 651 nucleotides from the transcription start site. To determine whether the transversion was involved in the etiology of the delayed speech, we analyzed the FMR1 mRNA from a lymphoblastoid cell line established from the proband. Northern analysis indicated that the polymorphism had no effect on splicing or the abundance of the FMR1 transcript. The proband's younger brother who also has this polymorphism, shows no sign of developmental delay at 1 year of age. Review of Southern analyses of >200 normal X chromosomes did not reveal any additional occurrences of this new Eag I site. Thus, this is a rare intronic polymorphism that has no apparent effect on FMR1 expression but which can potentially lead to a mistaken diagnosis of the deletion form of the fragile X syndrome. Although point mutations and deletions affecting FMR1 function have been found, they are exceedingly rare. Polymorphisms that alter the fragile X diagnostic pattern but have no functional consequence are probably more common. As screening for fragile X is broadened to include individuals with only a slightly increased risk of carrying an FMR1 mutation, the detection of polymorphisms with no etiological significance is likely to increase.

Program Nr: 1966 from 2002 ASHG Annual Meeting

Mutation screen of the glutamate decarboxylase 67 (GAD67) gene and haplotype association study to unipolar depression. *J. Lappalainen*^{1,2}, *G. Sanacora*¹, *H.R. Kranzler*³, *R. Malison*¹, *L.H. Price*⁴, *J. Krystal*^{1,2}, *J. Gelernter*^{1,2}. 1) Yale University, School of Medicine, Department of Psychiatry, New Haven, CT; 2) VA Connecticut Healthcare System, West Haven, CT; 3) University of Connecticut, Department of Psychiatry, Farmington, CT; 4) Brown University, Butler Hospital and Department of Psychiatry and Human Behavior, Providence, RI.

Recent studies using ¹H-MRI to quantify gamma-aminobutyric acid (GABA) in human brain have demonstrated lower concentrations of this neurotransmitter in major depression (Sanacora et al., 1999). The majority of brain GABA is synthesized by glutamate decarboxylase-67 (locus, GAD67). This enzyme is therefore in a critical position for brain GABA homeostasis. We screened the translated regions of the GAD67 gene (16 exons) for mutations using SSCP and/or dHPLC in a sample of 43 individuals with major depression. We identified eight variants (5 base substitutions, 2 insertion/deletions and 1 tandem repeat). Three SNPs, located in the 5' non-coding, intron 8 and 3' non-coding regions of the gene, were genotyped in 103 European-American (EA) subjects with depression and 125 EA psychiatrically screened controls. The frequency of the rarer allele of the SNPs was > 20%. Linkage disequilibrium (LD) and haplotype frequencies were estimated using the 3LOCUS program (Long et al., 1995). Marker and haplotype frequencies were not significantly different between the cases and controls. Significant LD was observed between the 3' non-coding region and intron 8 SNP. These results start to establish a haplotype map for GAD67, and suggest that the GAD67 gene is not a major locus for predisposition to major depression.

References: Sanacora et al., *Arch Gen Psychiatry* 56: 1043-1047, 1999, Long et al., *Am J Hum Genet* 56: 799-810, 1995.

Genotyping of the HTTLPR Ins/Del by dHPLC followed by Association/Linkage Disequilibrium to Behavior in a Southwest American Indian Isolate. X. HU, R. W. ROBIN, J. TAUBMAN, D. GOLDMAN. Lab of Neurogenetic, NIAAA/NIH, ROCKVILLE, MD.

BACKGROUND: Serotonin is pharmacobehaviorally implicated in anxiety/dysphoria and impulse control, both of which are genetically influenced. Serotonin's action as a neurotransmitter is terminated primarily by reuptake via the serotonin transporter (5-HTT). HTTLPR, a common 44bp ins/del polymorphism located in the 5' regulatory region of the gene, alters in vitro transcription and in vivo expression of the transporter. Linkage of this polymorphism to behaviors, particularly anxiety, has been reported. However, except for a previous sib-pair linkage in Finnish sibpairs, findings have been complicated by possibilities for ethnic stratification and genotyping errors inherent to gel-based genotyping. **METHODS:** We evaluated the relationship of HTTLPR to dimensionally measured anxiety and categorically assessed psychopathology in 582 Southwestern American Indians derived from inter-related pedigrees. dHPLC-based genotyping was performed by PCR of the GC rich region in the presence of 7-deaza-dGTP followed by automated analysis on a WAVE (Transgenomic, Inc., Omaha, NE) with a DNASep column and using 15 minute runs. Genotyping results were validated by comparison to genotypes from polyacrylamide gel. **RESULTS:** dHPLC is an efficient and highly accurate method for large-scale genotyping of this ins/del polymorphism. The transmission disequilibrium test (TDT) revealed the s allele was preferentially transmitted to affected individuals with alcoholism, posttraumatic stress disorder, anxiety, phobia, and major depression. **CONCLUSION:** These findings in an American Indian isolate support a role for HTTLPR in differential vulnerability to psychiatric disorders.

Program Nr: 1968 from 2002 ASHG Annual Meeting

Genetic risk detection using logistic regression analysis incorporating liability class applied in Alzheimer's disease. *K. Kamino¹, T. Kida¹, T. Miki², M. Takeda¹*. 1) Dept of Post-Genomics and Diseases, Osaka Univ. Grad. Sch. Med., Suita, Osaka, Japan; 2) Dept of Geriatric Medicine, Ehime Univ. Sch. Med., Ehime, Japan.

Genetic analysis of late-onset disease, especially Alzheimer's disease, is often difficult to replicate the positive association. This problem is partly due to disease penetrance. To solve this problem, we tested logistic regression with sex and apolipoprotein E allele with correction by liability class. We examined this statistics and found the risk for Alzheimer's disease in LDL receptor related protein (LRP), mitochondrial aldehyde dehydrogenase (ALDH2). Using this method, a polymorphism located at the short arm of chromosome 12 was also found to associate with Alzheimer's disease. This method will be useful to find the genetic risk sensitively .

The TGFb1 -509C/T promoter gene polymorphism as a risk factor for IgA nephropathy (IgAN)(Berger's disease). A. Turco¹, M. Gomez-Lira¹, M. Brugnara¹, B. Brezzi², A. Lupo², G. Gambaro³, F. Anglani³, D. Del Prete³, G. Maschio². 1) Dept of Mother & Child & Genetics, University of Verona School of Medicine, Verona, Italy; 2) Division of Medical Nephrology, Univ of Verona, Italy; 3) Dept of Medical & Surgical Sci, Nephrology Division, Univ of Padova, Padua, Italy.

TGFb1 is a cytokine that plays a critical role in the pathogenesis of glomerular damage by affecting cellular proliferation and matrix metabolism. In addition, it operates as an IgA-class switching factor, and its overproduction could also be etiologically involved in the individual susceptibility to IgAN, the commonest glomerulonephritis worldwide. Two TGFb1 gene polymorphisms, a C/T substitution at position -509 of the promoter region, and a T/C transition in exon 1 (cod10), are known to be associated with cardiovascular disease, nephritis, and with circulating protein levels. In this study we evaluated the possible association between these polymorphisms and the IgAN phenotype. 93 unrelated Italian patients and 100, sex matched healthy individuals from the same population were analyzed. Genotyping was performed by genomic DNA polymerase chain reaction (PCR), followed by restriction analysis. The results showed that the -509T variant was more frequent in IgAN patients than in controls, with allele frequencies of T=97/186 (53%) and T=75/200 (37%) respectively (p=0,0035). Genotype distributions were TT:CT:CC=22(24%); 53(57%); 18(19%) in IgA patients and TT:CT:CC= 14 (14%):47(47%):39(39%) in controls (p=0,003). The cod10 polymorphism showed no statistically significant differences between IgAN patients and controls. In summary, the presence of the -509T variant in the TGFb1 gene in our population appears to be a significant risk factor for the development of IgAN (OR=1,82-IC=98%-1,27 < OR <2,8).

Program Nr: 1970 from 2002 ASHG Annual Meeting

The *FMRI* Premutation Carrier And The Premature Ovarian Failure Phenotype. *A.K. Sullivan, L.J. Shubeck, E.G. Allen, W. He, J.J. Paquin, M.L. Leslie, L.H. Scott, S.L. Sherman.* Dept Human Genetics, Emory University, Atlanta, GA.

Premature ovarian failure (POF), defined as the cessation of menses before the age of 40, occurs in 1 in every 100 women in the general population. Approximately 20% of female *FMRI* premutation carriers experience POF. To date, the cause of POF in premutation carriers is unknown. In our study, we will evaluate whether *FMRI* CGG repeat size and/or parental origin is associated with increased risk for POF. Currently, we have obtained reproductive histories on 304 women ascertained through either families with the fragile X syndrome or the general population. We have confirmed *FMRI* CGG repeat size information on 214 of these women: 54 women have 61-199 repeats, 76 women have 41-60 repeats, and 84 women have <41 repeats. For those women with a *FMRI* CGG repeat size >40 repeats, parental origin information has been determined for 70 women. Follicle-stimulating hormone and luteinizing hormone levels have been measured on 89 women. Using linear and logistic regression analyses, we want to examine 1) if there is an association of age at menopause and/or POF with *FMRI* repeat size and 2) if parental origin of the large repeat size allele affects the risk for POF. If an association is found for repeat size and POF, we will determine the minimum number of repeats that increases the risk for POF. Preliminary data suggests that repeat size and parental origin may both play a role in the timing of menopause.

Mutation screening of candidate genes in congenital central hypoventilation syndrome. *I. Matera¹, T. Bachetti¹, R. Cinti¹, G. Santamaria¹, F. Mosca², F. Morandi³, M. Motta⁴, G. Ottonello⁵, R. Piumelli⁶, J.G. Schober⁷, C. Ghizzi⁸, M.P. Villa⁹, R. Ravazzolo¹, I. Ceccherini¹.* 1) Lab.Genetica Molecolare, G. Gaslini, Genova, Italy; 2) Dip. Neonatologia, Ist.Clin. di Perfezionamento, Milano, Italia; 3) U.O. Pediatria, Osp. Sacra Famiglia, Como, Italia; 4) U.O. Terapia Int. Neonatale, Spedali Civili, Brescia, Italia; 5) U.O. Anestesia e Rianimazione, Ist. G.Gaslini, Genova, Italia; 6) U.O. Terapia Int. Neonatale, Osp. Meyer, Firenze, Italia; 7) Kinderarzt-Kinderkardiologe-Neonatologe, Kinderklinik, Munchen, Germany; 8) U.O. Terapia Int. Pediatrica, Osp. Civile Maggiore, Verona, Italia; 9) Dip. Pediatria, Univ. La Sapienza, Roma, Italia.

Congenital central hypoventilation syndrome (CCHS, MIM 209880) is a rare neonatal disorder characterized by a failure of autonomic respiratory control. CCHS is frequently associated with Hirschsprung disease and tumors of neural crest origin. This has suggested a common molecular pathogenesis sustained by defect during the development of neural crest derived cells. We have already shown that the homeobox gene *RNX* is not mutated in our CCHS patients. The transcription factors *Mash1*, *Phox2a* and *Phox2b* are involved in the developing and specification of the autonomic nervous system. The *RET* proto-oncogene is normally expressed in cells of neural crest origin. On the basis of these observations, these genes were considered as candidate genes for CCHS. We have performed mutation screening by using denaturing high performance liquid chromatography and direct sequencing. No causative missense mutation of the entire coding region of the *ASH1* and *PHOX2B* genes was detected in any of the 19 CCHS index cases. *PHOX2A* and *RET* genes are under examination. We have also determined allele frequencies for five *RET* single-nucleotide polymorphisms in the CCHS patients. We found an association of some of these polymorphic *RET* variants with CCHS. This association suggests that this gene could contribute to the etiology of this disease, thus confirming a polygenic pattern of inheritance in CCHS. The search of additional candidate genes should be pointed toward genes involved in other neurocristopathies and/or in autonomic nervous system dysfunction.

SCA genotypes in Italian families with autosomal dominant cerebellar ataxia (ADCA). *C. Gellera*¹, *A. Brusco*², *A. Castucci*¹, *D. Vacca*¹, *C. Cagnoli*², *A. Saluto*², *C. Michelotto*², *C. Mariotti*¹, *S. DiDonato*¹, *F. Taroni*¹. 1) Div. Biochemistry & Genetics, Ist. Nazionale Neurologico C Besta, Milan, Italy; 2) Dept. Genetics, Biology & Biochemistry, University of Turin, Italy.

ADCA are a heterogeneous group of hereditary neurodegenerative disorders, primarily characterized by progressive cerebellar ataxia and clinically classified in different subgroups (ADCA I, IV). In the past few years, several genes (SCA genes) with different chromosomal localization were shown to be involved in the pathogenesis of these diseases, although the functions of most of the corresponding proteins are still unknown. The large majority of mutated genes harbor in the ORF an abnormal CAG expansion coding for a polyQ stretch; hence, a common pathogenic mechanism has been hypothesized in these disorders. Molecular investigations allowed genetic subgrouping of ADCA into spinocerebellar ataxias 1-17 (SCA1-17). SCA1, SCA2, SCA3, SCA6, SCA7, SCA12 and SCA17 are characterized by CAG repeat expansions while SCA8 is due to CTG enlarged repeats in the 3'UTR of a transcribed RNA and SCA10 is associated with the expansion of an intronic pentanucleotide (ATTCT) repeat. The other SCAs have been mapped by linkage analysis but the corresponding genes and molecular lesions have not been identified yet. We have clinically and genetically evaluated 172 Italian ADCA families. SCA1 and SCA2 were found to be the most common genotypes, accounting for 31% (54 families), and 36% (62 families) of cases, respectively. SCA3 (2 families), SCA6 (2 families), SCA7 (4 families), SCA8 (2 families), SCA17 (2 families) and DRPLA (3 families) were much less frequent. The remaining 41 families (24%) were found to be negative also for the recently identified mutations in SCA10, and SCA12 genes. The aim of this study is to report ADCA families presenting with rare genotypes in Italy and to select ADCA families negative for the known SCA genes in order to identify new SCA genes. Families with rare genotypes in Italy (i.e. SCA3, SCA6, SCA7, SCA8, SCA17 and DRPLA) have been closely examined either clinically and genetically.

Oxidative stress and apoptosis in the cellular model of Machado-Joseph Disease. *M. Hsieh¹, H.F. Tsai², S.Y. Li¹.* 1) Dept Life Sci, Chung Shan Med University, Taichung, Taiwan; 2) Institute of Medicine, Chung Shan Med University, Taichung, Taiwan, Republic of China.

Machado-Joseph disease (MJD) is an autosomal dominant spinocerebellar degeneration characterized by a wide range of clinical manifestations. Unstable CAG trinucleotide repeat expansion in MJD gene on long arm of chromosome 14 has been identified as the pathologic mutation of MJD. Because MJD is typically late-onset neurodegenerative disorder, we hypothesize that oxidative stress and apoptosis are contributory factors for the pathogenesis of this disease. In this study, we utilized neural SK-N-SH cells stably transfected with HA-tagged full-length MJD with 78 polyglutamine repeats to examine the effects of polyglutamine expansion on protein aggregation and cell survival under different stress. Our result showed that less than one percent of the mutant cells containing nuclear aggregates under basal condition. In addition, t-butyl hydroperoxide and various pro-apoptotic agents were used to assess the tolerance of the mutant cells. Cell Viability was determined by MTS assay. Mutant MJD cells were more sensitive to low concentration of t-butyl hydroperoxide (1 mM to 3 mM) than the normal cells without expanded MJD. We demonstrated that the ratio of GSH/GSSG increased about 4-fold in mutant MJD cells and the enzyme activities of Glutathione peroxidase and Glutathione reductase remained only 40% of those of the normal cells after 12-hour treatment of 1 mM t-BH. On the other hand, mutant MJD cells treated with 1 mM staurosporine showed significant decreased Bcl-2 level when compared to the normal cells. It is consistent to our observation that cells expressing mutant MJD were more sensitive to staurosporine-induced apoptosis. Taken together, MJD cells are more susceptible to the toxic insults than the normal cells without expanded MJD. We demonstrated that expression of full-length mutant MJD does not dramatically elevate protein aggregate formation under basal condition, but does significantly impair the ability of the cell viability to respond to stress and alter the redox status of the cellular proteins, which may lead to increase stress-induced cell death following stress.

Transglutaminase cross-linking of androgen receptor could inhibit the proteasome: possible pathogenic implications for spinobulbar muscular atrophy. *L.M. Mandrusiak^{1, 2}, L.K. Beitel¹, Y.A. El-haji^{1, 2}, T.C. Scanlon^{1,2}, C. Alvarado¹, B. Gottlieb^{1, 4}, M.A. Trifiro^{1, 2, 3}.* 1) Lady Davis Institute for Medical Research, Sir Mortimer B. Davis-Jewish General Hospital, Montreal, Canada; 2) Department of Human Genetics, McGill University, Montreal, Canada; 3) Department of Medicine, McGill University, Montreal, Canada; 4) Department of Biology, John Abbott College, Montreal, Canada.

Several different proteins bearing long polyglutamine (polyGln) tracts are known to cause neurodegenerative diseases by mechanisms that may involve a toxic gain of function. All these diseases are characterized by intracellular insoluble protein aggregates containing the Gln-expanded protein. The aggregates are ubiquitinated, but resistant to degradation by the proteasome. Expansions of the polyGln tract of the human androgen receptor (AR) cause spinobulbar muscular atrophy (SBMA). One established property of Gln residues is their ability to act as an amine acceptor in a transglutaminase-catalyzed reaction, resulting in a proteolytically resistant glutamyl-lysine cross-link. Transglutaminase (TG) is a calcium-dependent enzyme that naturally occurs *in vivo*. We have found that bacterially expressed AR is a substrate of guinea pig liver tissue TG *in vitro*. Both GST-AR fusion proteins and thrombin cleaved (to remove the GST) proteins were shown to react with TG. Western blots of the proteins following incubation with TG demonstrate that several different epitopes of the AR (1C2, polyGln tract, 441, amino acids 301-321, PG21, amino acids 1-20) appear to be lost. We propose that this is due to TG cross-linking of the AR, which interferes with antibody recognition. Interestingly, both intermolecular and intramolecular bonds appear to be formed. When AR with polyGln tracts of varying length were analyzed, it was found that expanded (50 Gln) AR has a greater propensity to form intermolecular bonds than the normal length protein. TG cross-linked intermolecularly bonded AR is hypothesized to decrease proteasome function by clogging the proteasome pore. The inhibited ubiquitin proteasome pathway could contribute to the selective cell death seen in the SBMA phenotype.

Histone Hyperacetylation and Reactivation of the Fragile X Syndrome Gene. *G. Neri*¹, *E. Tabolacci*¹, *R. Pietrobono*¹, *M.G. Pomponi*¹, *P. Chiurazzi*². 1) Institute of Medical Genetics, Catholic University, Rome, Italy; 2) Department of Pediatrics, University Hospital, Messina, Italy.

Fragile X syndrome is the most common inherited mental retardation condition and is almost always caused by the expansion of a CGG repeat in the promoter region of the *FMR1* gene, which is then abnormally methylated and becomes inactive. We demonstrated that treatment of fragile X lymphoblasts with the DNA demethylating drug 5-azadeoxycytidine (5-azadC) can reactivate the *FMR1* gene (Hum Mol Genet 7:109-113, 1998). Histone hyperacetylating drugs such as 4-phenylbutyrate and sodium butyrate alone induce only a modest reactivation, but strongly reinforce the effect of 5-azadC (Hum Mol Genet 8:2317-2323, 1999). We then investigated the methylation status of each CpG site in the *FMR1* promoter by bisulphite sequencing and proved that 5-azadC leads to a nearly complete demethylation of the promoter (Nucl Acids Res, in press). We went on to investigate the histone acetylation status using chromatin immunoprecipitation with polyclonal antiacetylated-H4 and antiacetylated-H3 antibodies (Upstate Biotechnology) and real-time fluorescent PCR with TaqMan probes specific for the *FMR1* and *HPRT* genes. We treated fragile X and normal lymphoblastoid cell lines with different concentrations of sodium butyrate and observed an increase of histone H4 acetylation and lesser increase (and sometimes a decrease) of histone H3 acetylation in treated cells. We found a similar increase in H4 acetylation after treatment of fragile X cells with 5-azadC alone, confirming that DNA demethylation eventually causes relaxation of chromatin conformation and histone reacetylation. We then explored the potential acetylating action of acetyl-L-carnitine (ALC), an FDA-approved drug which resembles butyrate. Preliminary results with ALC alone showed an increase of the immunoprecipitated DNA only from treated cells, although not clearly correlated with *FMR1* gene reactivation. Supported by grants from FRAXA Foundation, Sigma-Tau and MIUR, Italy.

Y Chromosome microdeletions in oligozoospermic and azoospermic idiopathic male patients. *F.E. Orkunoglu¹, H. Soydan², Y. Ozgok², Y. Saglam³, A. Sengul⁴, M. Dayanc², N. Imirzalioglu¹.* 1) Medical Genetics, Gulhane School of Medicine, Ankara, Turkey; 2) Urology, Gulhane School of Medicine, Ankara, Turkey; 3) University of Kadir Has, Dept Med Biology and Genetics, Istanbul, Turkey; 4) Immunology, Gulhane School of Medicine, Ankara, Turkey.

Y chromosome microdeletions have been reported as a possible genetic factor for male infertility. Molecular analyses of azoospermic and oligozoospermic males have suggested the existence of deletions in the four functional regions of intervals 5 and 6 on the Y chromosome associated with spermatogenic failure. The aim of this study was screening of microdeletions in the four-azoospermia factor (AZF) regions on the Y chromosome in Turkish infertile male patients with idiopathic azoospermia and oligozoospermia. 18 known sequence tagged sites (STS) primer pairs were tested in four multiplex PCR sets to detect Y microdeletions that associated with male infertility in patients and controls that are fathers. Microdeletions or gene-specific deletions were not detected in normospermic control subjects or in subfertile men with a sperm count of more than 1 10⁶/mL. Deletions of the AZFc region were detected in 3% of individuals with idiopathic azoospermia or oligozoospermia. AZFc may be the most important subregion to screen infertility. In addition, intact AZFa and AZFb subregions may be important for the presence of germ cells. As a result, the frequency of Y microdeletions in the Turkish population is similar to that from other countries.

Transmission of full mutation alleles from premutation males to daughters. *S.L. Nolin¹, C.S. Dobkin¹, L. Gane², X.H. Ding¹, G.E. Houck, Jr.¹, A.D. Gargano¹, A. Glicksman¹, S.Y. Li¹, W.T. Brown¹.* 1) NYS Institute for Basic Research in Developmental Disabilities, Staten Island, NY; 2) UC Davis M.I.N.D. Institute, Sacramento, CA.

In fragile X, the dramatic expansion in CGG repeat size to more than 200 in a full mutation is thought to occur exclusively on transmission from premutation females to their offspring. Males with premutation or full mutation alleles are observed to have premutation-size repeats in their sperm and transmit premutation alleles to their daughters. We report here two unrelated cases of transmission of full mutation-size repeats from premutation males to their daughters. In the first family, the FMR1 CGG repeat region was examined in DNA from the mother, father and three daughters. While the mother was found not to be a carrier for fragile X, the father carried a premutation allele of 105 repeats and two of the daughters carried alleles of 100 and 95 repeats. PCR analysis of a third daughter revealed a predominant band of 145 repeats and a series of bands extending into the full mutation range. Southern analysis revealed a series of unmethylated fragments that extended from premutation into the full mutation range, in addition to the bands representing the normal allele. In the second case, PCR and Southern analysis of one daughter of a premutation male revealed a series of repeats from 135 to >200. The unusual molecular patterns of the two females in these two families were notable for the unmethylated fragments observed in Southern analysis. This pattern is not associated with females carrying full mutation alleles that are maternally transmitted. Two similar cases have been reported elsewhere (Bridge et al., *Gen in Med* 1: 49, 23A, 1999; Ventura et al., *AJHG* 65:A471, 1999). We suggest a paternally transmitted allele may expand to a full mutation that has a distinctive molecular pattern. While this expansion leads to somatic instability typical of maternally transmitted full mutations, the size of the expansion is limited and does not appear to lead to aberrant methylation. This suggests that paternal transmission of the fragile X full mutation occurs by a unique mechanism.

A cerebellar FMR1 riboCGG binding protein. *T.C. Rosser, T.R. Johnson, S.T. Warren.* Howard Hughes Medical Institute and Department of Human Genetics, Emory University School of Medicine, Atlanta, GA.

Fragile X syndrome, a common form of mental retardation, results from an expanded CGG repeat in the 5' UTR of the FMR1 gene. Full mutations (>200 repeats) elicit silencing of the FMR1 locus and the absence of the FMR1 protein, FMRP. Premutation alleles (60-200 repeats) appear to be nonpenetrant for the full mutation phenotype, although such carriers have a higher incidence of other phenotypes. Premutation females have an increased incidence (24%) of premature ovarian failure when compared to the normal population (1%). Male premutation carriers may show an age-dependent progressive tremor and motor/gait problems. This is combined with cognitive decline associated with accumulations of inclusion bodies in cerebellar neurons and astrocytes. Since the premutation phenotypes are distinctive from those of fragile X syndrome, due to the absence of FMRP, it is possible that these phenotypes are RNA-mediated. We propose a mechanism similar to that described for another triplet repeat disorder, myotonic dystrophy (DM). In DM, a portion of the phenotype is thought to be due to the sequestering of a RNA-binding protein on the expanded rCUG-repeat. We hypothesize here that another RNA-binding protein, interacting with the rCGG-repeat of the FMR1 message, is similarly sequestered from its normal function(s) in premutation carriers. In order to identify such a rCGG-BP, we used in vitro transcription to fluorescently label RNA containing a CGG₍₁₀₅₎ repeat. We have shown by gel shift assays, the presence of a rCGG repeat binding protein in the cerebella of mice. Binding could be competed off with unlabelled rCGG but not with other RNA transcripts. Most interesting, this gel shift was not equivalent between male and female cerebellar lysates, suggesting this protein may be male-biased. Efforts currently are underway to purify the rCGG-BP and establish its identity by mass spectrometry.

CpG Methylation Modifies the Genetic Stability of Various Repeat Sequences. *C.E. Pearson^{1,2}, K. Nichol¹*. 1) Dept Genetics/Genomic Biol, Hospital for Sick Children, Toronto, Ontario, Canada; 2) Dept Molecular & Medical Genetics, University of Toronto, Ontario, Canada.

Numerous disease states are associated with both repeat instability and aberrant CpG methylation, including fragile X mental retardation, myotonic dystrophy and specific cancers. The genetic stability of tandemly repeated DNAs is affected by repeat sequence, tract length, tract purity and replication direction. Alterations in DNA methylation status are thought to influence many processes of mutagenesis. Using bacterial and primate cell systems we have determined the effect of CpG methylation on the genetic stability of cloned di-, tri-, penta-nucleotide and minisatellite repeated DNA sequences. Depending upon the repeat sequence, methylation can significantly enhance or reduce its genetic stability. This effect was evident when repeat tracts were replicated from either direction. Unexpectedly, methylation of adjacent sequences altered the stability of contiguous repeat sequences void of methylatable sites. Of the seven repeat sequences investigated, methylation stabilized five, de-stabilized one and had no effect on another. Thus, while methylation generally stabilized repeat tracts, its influence depended upon the sequence of the repeat. The current results lend support to the notion that the biological consequences of CpG methylation may be effected through local alterations of DNA structure as well as through direct protein-DNA interactions. Since CpG methylation is highly regulated in a tissue- and development-specific manner its alteration may contribute to mutagenesis and disease etiology. Furthermore, methylation in bacteria may have technical applications to fill-in gaps or genomic "blind-spots" within genome draft sequences specifically enabling the analyses of DNA sequences that have been recalcitrant to isolation due to their extreme instability.

Bimodal, length-dependent, somatic instability of the GAA triplet-repeat sequence in Friedreich ataxia. R.

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Friedreich ataxia is most commonly caused by large expansions of a GAA triplet-repeat sequence in the first intron of the *FRDA* gene. We have used small-pool PCR to investigate somatic instability of the GAA triplet-repeat sequence in peripheral leukocytes *in vivo*. We analyzed 15 normal alleles ranging from 8 - 25 triplets, one premutation allele with 44 triplets and 12 disease-causing expanded (E) alleles with 241 - 1105 triplets. Analysis of 7190 individual *FRDA* molecules derived from genomes containing E alleles showed a length-dependent increase in somatic variability with mutation loads ranging from 47 - 78%. E alleles with >500 triplets were four-fold more likely to undergo large contractions, 5% of which involved >50% of the initial allele length and 0.29% involved complete reversion to the normal / premutation size range. Conversely, E alleles containing <500 triplets showed an expansion bias. No somatic variability was detected in over 6000 *FRDA* molecules derived from carriers of normal alleles. The premutation allele with 44 uninterrupted GAA triplets was found to be unstable, ranging in size from 6 - 113 triplets. This establishes the threshold for somatic instability to between 26 and 44 uninterrupted GAA triplets in peripheral leukocytes *in vivo*, very similar to the observed threshold for expansions during germline transmission, and approximately the size of a eukaryotic Okazaki fragment. Consistent with the observed tendency for shorter E alleles (<500 triplets) to undergo expansion mutations, the premutation allele also showed a strong expansion bias. 6% of the 2304 *FRDA* molecules analyzed differed significantly from the initial allele length; 46% were expansions of 14 - 36%, but only 2% of the altered bands comprised similar sized contractions. We propose a replication-based model to explain the observed somatic instability.

Maternal uniparental isodisomy 20 in a fetus with trisomy 20 mosaicism: clinical, cytogenetic and molecular

analysis. *V. Velissariou¹, T. Antoniadi¹, J. Gyftodimou², K. Bakou¹, M. Grigoriadou², S. Christopoulou¹, A. Hatzipoulou¹, J. Donoghue¹, P. Karatzis³, E. Katsarou⁴, M.B. Petersen².* 1) Department of Genetics and Molecular Biology, Mitera Maternity and Surgical Center, Maroussi, Greece; 2) Department of Genetics, Institute of Child Health, Athens, Greece; 3) IVF Unit, Mitera Maternity and Surgical Center, Maroussi, Greece; 4) Department of Neurology, "P. & A. Kyriakou" Children's Hospital, Athens, Greece.

The clinical significance of trisomy 20 mosaicism detected prenatally remains uncertain, due to the rarity of liveborn cases with inconsistent clinical findings and lack of long-term follow-up and outcome. We describe a case of true trisomy 20 mosaicism in a liveborn girl with maternal uniparental isodisomy of chromosome 20 in the diploid blood cells. Trisomy 20 mosaicism was originally detected in amniotic fluid (98%) and was confirmed in the term placenta (100%), as well as in blood (10%) and urine sediment (100%) of the newborn. There was intrauterine and postnatal growth retardation but otherwise the newborn manifested no gross abnormalities. At 9 months of age moderate psychomotor retardation, central hypotonia with peripheral hypertonia, numerous minor morphogenetic variants, marked kyphosis, and extensive Mongolian spot were observed. To our knowledge this represents the first case of trisomy 20 mosaicism detected prenatally and confirmed in different tissues of the newborn, where uniparental disomy was demonstrated in the diploid cell line. It is discussed whether uniparental disomy for chromosome 20 in the diploid tissues of mosaic trisomy 20 fetuses can be of clinical significance.

Characterisation of a Microdeletion in Xp11.23 Cosegregating in a Small Family with X-Linked Retinitis Pigmentosa (RP2) and Psychosis. *D.L. Thiselton^{1,3}, O. Brandau², A. Meindl², B.P. Riley³, K.S. Kendler³, L. Van Maldergem⁴, A.J. Hardcastle¹.* 1) Dept. of Molecular Genetics, Institute of Ophthalmology, UCL, London, UK; 2) Abteilung fuer Padiatrische Genet der LMU, Munich, Germany; 3) Dept. of Psychiatry, VCU, Richmond, VA, USA; 4) Dept. of Medical Genetics, Institute of Pathology, Gerpennes, Belgium.

As part of a targeted STS-deletion screen of XLRP families to pinpoint the genomic critical region encompassing the RP2 gene, a family was identified in which 2 brothers, both affected with RP, shared a common deletion in Xp11.23. PCR analysis using a dense array of STS markers positioned onto a comprehensive physical map established that the deletion spanned ~700kb between markers DXS1408 and stsG9154, and includes the *RP2* gene. In addition to RP, the brothers share a history of psychosis, the more severely affected having a DSM-IV diagnosis of paranoid schizophrenia.

By analysis of genomic sequence data, we have generated novel STSs to more precisely delineate the deletion endpoints, and used both RT-PCR and bioinformatic tools to functionally annotate the deleted interval and identify genes and pseudogenes located within it. Long-range PCR, cloning and sequencing is now underway to determine the exact deletion breakpoint.

Several reports have provided evidence of a schizophrenia susceptibility locus on Xp11. The family described here may offer cytogenetic support for such a notion and assist in the search for associated genetic variants. Studies are in progress to more definitively ascertain the psychiatric phenotype in this family, to develop a characterized SNP map of the interval, and to examine the affected genes for mutations or sequence variants which may be associated with a similar disorder in relevant patient cohorts.

Genomic rearrangements resulting in *PLP1* deletion occur by non-homologous end-joining and cause different dysmyelinating phenotypes in males and females. *K. Inoue*¹, *H. Osaka*³, *V.C. Thurston*⁴, *J.T.R. Clarke*⁵, *A. Yoneyama*⁶, *L. Rosenbarker*¹, *T.D. Bird*⁷, *M.E. Hodes*⁴, *L.G. Shaffer*¹, *J.R. Lupski*^{1,2}. 1) Dept Molec & Human Genetics, Baylor Col Medicine, Houston, TX; 2) Dept Pediatrics, Baylor Col Medicine, Houston, TX; 3) Dept Neurodegenerative Diseases, National Center for Neuroscience, NCNP, Tokyo; 4) Dept Med and Molec Genetics, Indiana Univ, Indianapolis, IN; 5) Dept Genetics, The Hospital for Sick Children, Toronto, Ontario; 6) Dept Pediatrics, National Rehabilitation Center for Disabled Children, Tokyo; 7) Dept Neurology, and Medicine, Univ of Washington, Seattle, WA.

Duplication of *PLP1* is responsible for the majority of patients with Pelizaeus-Merzbacher disease (PMD), while deletion of *PLP1* is infrequent. Genomic mechanisms for these submicroscopic chromosomal rearrangements remain unknown. We identified two families with *PLP1* deletion that arose from two different genomic recombination mechanisms. In one family, *PLP1* deletion resulted from a maternal balanced submicroscopic interstitial translocation of the entire *PLP1* gene to the telomere of the chromosome 19 (19qtel). *PLP1* on the 19qtel is probably inactive by virtue of a position effect, because a healthy male sibling carries the same 19qtel(*PLP1*) along with a normal X chromosome. In the other family, the deletion occurred during meiosis of a maternal grandfather via sister chromatid exchange. Genomic mapping of the deleted segments in three *PLP1* deletion families reveals that the deletions are smaller than most of the *PLP1* duplications and involve fewer genes. We hypothesize that the deletion is infrequent because larger deletions may be deleterious. Analyses of the DNA sequence at the recombination junction reveal *Alu-Alu* recombination in the translocation family. In the other two families, no homologous sequence was found flanking the breakpoints, but the distal breakpoints were embedded in low copy repeats (LCR-PMD), suggesting the potential involvement of LCR-PMD in these rearrangements. In one family, junction sequences revealed a complex recombination event. Our data suggest that *PLP1* deletions are not mediated by non-allelic homologous recombination at LCR, but rather non-homologous end-joining.

Several Novel Androgen Receptor Gene Mutations associated with Diverse Presentations of Androgen

Insensitivity Syndrome. *O.T. Mueller*^{1,3}, *H. Mason-Suares*^{1,2}, *A.W. Root*^{1,3}. 1) Molecular Genetics Laboratory, All Children's Hosp, St Petersburg, FL; 2) Eckerd College, St. Petersburg, FL; 3) University of South Florida College of Medicine, Tampa, FL.

Androgen Insensitivity Syndrome (AIS) presents in X,Y individuals as either complete (C), partial (P) or mild (M) forms and is associated with mutations in the androgen receptor (AR) gene. Individuals with CAIS have external female genitalia but have testes and are infertile. Partial AIS presents as individuals with ambiguous external genitalia or males with microphallus or hypospadias. Mild AIS presents as undervirilized males with gynecomastia. We determined the DNA sequence of the eight coding exons of the androgen receptor gene in 11 individuals referred for possible AIS. Five cases presented with ambiguous genital (possible PAIS) and six cases presented with female external genitalia (possible CAIS). Data on internal genitalia and steroid levels are available from some, but not all patients. All patients have an X,Y karyotype and a grossly normal SRY gene (not sequenced). Only one of the PAIS cases was found with a pathogenic mutation: a AGC->AGT synonymous change in codon 888 that introduces a cryptic splice donor sequence. This mutation was previously described and causes an alternate transcript skipping part of exon 8 and the 3'UTR. Normal length transcripts are also produced, explaining the partial phenotype. Three of the six cases of CAIS were found to have pathogenic mutations: one case with a previously described Asp to Ser change at codon 705 (N705S). Another CAIS case was discovered to have a C to A change at -59 bp from the start of the fourth exon. This intron change was not previously reported and it was not present in any of the other cases or 10 normal controls. The familial inheritance of this putative mutation will be presented to help establish its pathogenicity. A third case of CAIS was discovered to have a deletion of guanine in codon 870 that caused a frame shift revealing a premature stop triplet at codon 881. This is apparently one of the first descriptions of a frameshift deletion in the androgen-binding domain of this gene causing CAIS.

The Oral-facial-digital syndrome type 1 gene codes for human metanephric mesenchymal protein. *L. Romio¹, V. Wright¹, K. Price², P.J.D. Winyard², S. Malcolm¹, A.S. Woolf², S.A. Feather².* 1) Clinical & Molecular Genetics, Institute of Child Health UCL, London, UK; 2) Nephrourology Unit Institute of Child Health UCL, London, UK.

We recently identified OFD1, the gene responsible for the oral-facial-digital syndrome type 1, a cause of dominant polycystic kidney disease (PKD) and malformation of the face, oral cavity and digits. To date only a handful of mutations have been reported and nothing is known about the OFD1 protein. WAVE analysis and sequencing of the 23 OFD1 exons in further cases revealed several novel mutations, both in familial and sporadic cases, the majority leading to premature truncation of the protein. We analysed expression of OFD1 in first trimester normal human embryos: RT-PCR shows that OFD1 is widely expressed during development; with northern blot we could detect OFD1 mainly in brain, kidney, limb and tongue which are all organs affected by the disease. We raised a polyclonal antibody to a C-terminal epitope of the protein. In normal human embryos OFD1 immunolocalised to metanephric mesenchyme, oral mucosa, nasal and cranial cartilage and brain. We generated human renal mesenchymal cell lines and by western blot we detected a band of approximately 120kD, consistent with OFD1 size; a similar signal was also present in lysate of whole metanephroi. This is the first demonstration that OFD1 transcript and protein are expressed in early human development; based on the metanephric expression pattern OFD1 may play a role in differentiation of the renal mesenchymal lineage into nephrons.

A Novel Cell Model of the Polyglutamine Disease Spinal and Bulbar Muscular Atrophy. *J.L. Walcott^{1,2}, D.E. Merry²*. 1) Department of Pharmacology, University of Pennsylvania School of Medicine, Phila., PA; 2) Biochemistry and Molecular Pharmacology, Thomas Jefferson University, Phila., PA.

Spinal and bulbar muscular atrophy (SBMA) is one of a group of chronic progressive neurodegenerative diseases resulting from a polyglutamine repeat expansion. In SBMA patients, the polymorphic trinucleotide CAG repeat in exon 1 of the androgen receptor (AR) gene is increased, resulting in polyglutamine tract expansion. We have created a tetracycline inducible cell model of SBMA in PC12 cells expressing full-length androgen receptor with 112 repeats. Cells expressing highly expanded AR112 form ubiquitinated intranuclear inclusions containing the amino terminal portion of AR and heat shock proteins. The inclusions appear as distinct granular electron dense structures in the nucleus by electron microscopy. The formation of AR-containing inclusions is substantially increased with ligand treatment and correlates with decreased cell survival. This model mimics the formation of inclusions containing the amino terminal portion of AR and ubiquitin seen in patients and will serve as an excellent system for the study of SBMA pathogenesis and treatment.

Molecular modelling of CDH23 carrying missense mutations that cause DFNB12 strongly suggests impaired calcium-binding. A.P.M. de Brouwer^{1,2}, M. Roeters³, L.M. Astuto⁴, C.W.R.J. Cremers², F.P.M. Cremers¹, H. Kremer².
1) Department of Human Genetics, UMC St. Radboud, Nijmegen, The Netherlands; 2) Department of Otorhinolaryngology, UMC St. Radboud, Nijmegen, The Netherlands; 3) CMBI, University of Nijmegen, Nijmegen, The Netherlands; 4) Gene Marker Laboratory, Boystown National Research Hospital Omaha, NE, USA.

Mutations in the cadherin 23 gene (*CDH23*) cause recessive deafness DFNB12, Usher 1D and atypical Usher syndrome. DFNB12 is caused by missense mutations, whereas Usher 1D is caused by protein truncating mutations. Atypical Usher syndrome can be caused by both missense and truncating mutations. *CDH23* is part of the cadherin superfamily, of which the members are characterized by calcium-binding extracellular cadherin (EC) repeats that mediate cell-to-cell contact. *CDH23* is expressed in the inner and outer hair cells of the cochlea. The stereocilia organization of these hair cells is disrupted in *waltzer* (*v*) mice, the mouse model for Usher 1D. Primarily based upon these observations, *CDH23* is proposed to be part of the tip links or of the lateral links between the stereocilia. We identified two new missense mutations in *CDH23* in a family with sensorineural hearing loss. The EC repeats of *CDH23* containing these mutations as well as seven other EC repeats with mutations described elsewhere were aligned, which suggested that the substituted amino acid residues are part of the highly conserved calcium-binding sites. Molecular modelling of the mutated EC repeats based on the structure of E-cadherin, clearly showed that calcium-binding is impaired. Since calcium provides rigidity to the elongated structure of cadherin molecules enabling homophilic lateral interactions, these mutations are likely to impair interactions of *CDH23* molecules either with *CDH23* or with other proteins. By molecular modelling we computed the total length of the 27 EC domains from *CDH23* to be approximately 120 nm. This supports the suggestion that *CDH23* is a component of the lateral links, since the length is in the order of half the distance between neighbouring stereocilia in mouse.

Fine-mapping of the DFNA18 region and exclusion of ZNF9 as the disease gene. *D. Boensch^{1,3}, J. Schickel¹, R. Lang-Roth², C. Neumann¹, O. Witte³, A. Lamprecht-Dinnesen², T. Deufel¹.* 1) Institut fuer Klinische Chemie, Friedrich-Schiller Universitaet, Jena, Jena, Germany; 2) Poliklinik fr Phoniatrie und Paedaudiologie, Universitaet Muenster, Germany; 3) Klinik fuer Neurologie, Friedrich-Schiller Universitaet, Jena, Jena, Germany.

We have previously mapped a locus for non-syndromic autosomal dominant hearing loss, DFNA18, to a region on chromosome 3q22 overlapping the PROMM/DM2 locus. The majority of PROMM-pedigrees seem to map to the DM2 locus, and hearing loss has been described as one clinical feature in PROMM disease. This situation parallels that in myotonic dystrophy (DM1), localised on chromosome 19, where in some pedigrees hearing loss has even been reported as the only presenting symptom. Recently, a CCTG expansion (mean approximately 5000 repeats) within intron 1 of the zink finger protein 9 gene (ZNF9), segregating with the disease, has been identified in PROMM pedigrees mapped to the DM2 locus. The gene consists of five exons, four of which contain coding information for two alternatively spliced products. ZNF9, which is also known as cellular nucleic acid-binding protein (CNBP), contains 7 zinc-finger domains and has been implicated in the negative regulation of cholesterol biosynthesis as well as the modulation of the activity of human beta-myosin heavy chain gene (beta-MHC) by interaction with a repressor region; its developmental expression has been shown in *xenopus laevis*. We have excluded repeats and mutations within the ZNF9 gene in DFNA18 patients. After analysing further members of the pedigree, we were then able to fine-map the DFNA18 region which is now more telomeric, overlapping with DFNB15. Candidate genes, partially with cochlear expression, have been identified and sequencing is on the way.

Molecular analysis of the PDS gene in 16 families with Pendred syndrome. *D. Feldmann¹, F. Denoyelle², V. Duval¹, O. Messaz¹, R. Couderc¹, C. Petit³, E.N. Garabedian², S. Marlin⁴.* 1) Laboratoire de Biochimie, Hopital A. Trousseau, AP-HP, Paris, France; 2) Service d'ORL pediatrique, Hopital A. Trousseau, AP-HP, Paris, France; 3) Unite de genetique des deficits sensoriels, Institut Pasteur, Paris, France; 4) Unite de Genetique Clinique, Hopital A. Trousseau, AP-HP, Paris, France.

Pendred syndrome is an autosomal-recessive disorder characterized by congenital sensorineural hearing loss combined with goitre. It may account for as many as 10% of the cases of hereditary deafness. Mutations in PDS gene have been observed in patients with Pendred syndrome or with non syndromic deafness linked to 7q31 (DFNB4). We have studied the PDS gene in 16 unrelated families with Pendred syndrome. All the patients were prelingually deaf, with moderate or profound deafness. Cochlear malformations or a widened vestibular aqueduct was documented in 13/16. Goitre and or an abnormal perchlorate discharge test was present in all cases. We have developped a DGGE/sequencing method analysis of the entire coding part of the PDS gene. A large spectrum of mutations have been observed, but three mutations (Y530H, T416P, V138F) have been observed in respectively 12.5, 9.3 and 6.2% of all the mutated alleles. Three new mutations have been identified : S133X, IVS14+1G/A and S137P. The phenotype associated with these mutations will be described.

A deletion involving the GJB6 gene is not a frequent cause of nonsyndromic deafness in the Greek population. A.

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Mutations in the gene encoding the gap junction protein connexin 26 (GJB2) at the DFNB1 locus are responsible for up to 40% of all cases of nonsyndromic prelingual deafness in different populations. However, a fraction of patients with GJB2 mutations have only one mutant allele, and some familial cases with linkage to the DFNB1 locus but with no mutation in GJB2 have also been reported. Recently, a deletion involving the GJB6 gene encoding connexin 30, which is also located at the DFNB1 locus, was reported in a proportion of prelingual deafness patients heterozygous for a GJB2 mutation. Homozygosity for the GJB6 deletion was also reported in a few deafness patients. We therefore thought to screen for the GJB6 deletion in a population-based material of prelingual deafness from Greece. The material consisted of 210 unrelated Greek patients (45 familial/165 sporadic cases) with nonsyndromic, prelingual, sensorineural deafness from various regions of the country. Biallelic GJB2 mutations were detected in 70 of the patients (33.3%), whereas 6 patients were heterozygous for the 35delG mutation with no second mutation detected by sequencing of the entire coding region of the GJB2 gene. Screening for the GJB6 deletion was carried out in the 6 35delG/- heterozygotes and in 25 familial cases without GJB2 mutations, using a specific PCR assay to amplify the breakpoint junction of the 342-kb deletion involving GJB6 (NEJM 2002;346:243-9). The GJB6 deletion was not detected in our material. We conclude that the GJB6 deletion is not a frequent deafness mutation in the Greek population as previously described in Spanish, French and Ashkenazi Jewish populations.

Prestin, a cochlear motor protein, is defective in non-syndromic recessive deafness. *X.Z. Liu¹, X.M. Ouyang¹, X.J. Xia², J. Zheng³, L. Fang¹, A. Pandya², L.L. Du¹, D. Corey⁴, K.O. Katherine⁵, C. Petit⁶, R.J.H. Smith⁷, K.S. Arnos⁵, P. Dallos³, T. Balkany¹, W.E. Nance², Z.Y. Chen⁴.* 1) Dept Otolaryngology (D-48), Univ Miami, Miami, FL; 2) Dept Human Genetics, Medical College of Virginia of VCU, Richmond, Virginia; 3) Dept Communication Sciences and Disorders, Auditory Physiology Laboratory, Northwestern University, Evanston, Ill; 4) Neurology Department, Massachusetts General Hospital and Neurobiology Department, Harvard Medical School Boston, MA; 5) Dept Biology, Gallaudet University, 800 Florida Ave. NE Washington DC; 6) Unit de Gntique des Dfcits Sensoriels, CNRS URA 1968, Institut Pasteur, 25 rue du Dr Roux-75724 Paris; 7) Dept Otolaryngology, University of Iowa, Iowa City, IA.

Prestin, a membrane protein that is predominantly expressed in the outer hair cells (OHCs) of the cochlea, is a member of a newly recognized class of molecular motors. This family of genes encodes anion-transporter-related proteins, specifically, solute carrier family (SLC) 26. Nine human genes have been cloned so far, including SLC26A2, SLC26A3, SLC26A4, and SLC26A6. Mutations in SLC26A4 can cause Pendred syndrome as well as autosomal recessive non-syndromic deafness. The discrete localization of prestin expression in the OHCs makes it a strong candidate gene for a human deafness. Here we report the cloning and characterization of the human prestin gene, SLC26A5. SLC26A5 is composed of 20 exons with a coding region of 2232 bp, spanning a genomic region of over 70 kb on chromosome 7. Histochemical studies of mouse cochlear preparations showed immunostaining for prestin only in the OHCs. The expression profile of prestin was also studied through RT-PCR and northern blot. Moreover, we have identified a 5-UTR splice acceptor mutation (IVS2-2A>G) in this gene which is responsible for recessive non-syndromic deafness in two unrelated families, thereby assigning an essential function to prestin in the hearing process. Supported by NIH R01 DC05575.

Frequency of the Connexin 30 deletion (Cx30del) in deaf probands from the U.S. and its occurrence of *cis* with Connexin 26 (Cx26) mutations. *W. Nance*¹, *X. Xia*¹, *V. Gupta*¹, *K. Arnos*², *K. Welch*², *B. Landa*¹, *S. Dobrowski*³, *S. Blanton*¹, *A. Pandya*¹. 1) Dept Human Genetics, Virginia Commonwealth Univ, Richmond, VA; 2) Dept Biology, Gallaudet Univ, Washington, DC; 3) NeoGen, Pittsburgh, PA.

Profound deafness affects 1 in 1000 newborns, with 50% or more having a genetic etiology. In the U.S., mutations in the Cx26 (GJ2) gene are the most common genetic cause. Del Castillo et al. (NEJM 346:243-249, 2002) recently described the digenic transmission of deafness resulting from mutations of the Cx26 and Cx30 (GJB6) genes, located within ~400 kb region on 13q12 and are both expressed in the cochlea. Deafness results when a recessive Cx26 mutation is paired in trans with a 342 kb deletion involving the Cx30 gene. Homozygotes for the deletion are also deaf. Although a digenic interaction between the products of the two genes is suspected, it has not been possible to exclude the possibility that the deletion simply influences the activity of a 5' regulator of Cx26. To determine the frequency of Cx26/Cx30 deafness in the U.S., we screened 626 deaf probands from a large national DNA repository. Among 430 deaf probands with normal Cx26 alleles, 1 was homozygous and 4 heterozygous for the Cx30del; among 62 heterozygotes for pathologic Cx26 alleles, 11 Cx30del carriers were found. Of particular interest, 1 of 134 Cx26 homozygotes was also a Cx30del carrier and must have carried the two mutations in *cis*. The frequency of Cx30del in our deaf probands is 0.014, and 17.7% of those who were heterozygous for recessive Cx26 alleles also carried Cx30del which thus accounted for their deafness. We have observed a second possible example of the *cis* configuration in a proband who appeared to have inherited a dominant form of deafness. If *cis* mutations do cause hearing loss, it would indicate true digenic inheritance rather than a regulatory interaction between the two genes. It could also provide a plausible explanation for Cx26 alleles that appear to be dominant in some families and recessive in others. Cx30 is an important feature of Connexin deafness, but the fact that Cx30del explains a fraction of deaf Cx26 heterozygotes clearly suggests the existence of other epistatic loci.

Suppression subtraction hybridization as a tool to identify cochlea-specific genes. *M.W.J. Luijendijk¹, H. Kremer², G. van Duijnhoven¹, T.R.J. van de Pol¹, H.G. Brunner¹, F.P.M. Cremers¹.* 1) Human Genetics, Univ Med Ctr Nijmegen, Nijmegen, The Netherlands; 2) Otorhinolaryngology, Univ Med Ctr Nijmegen, Nijmegen, The Netherlands.

To identify cochlea-specific genes, a cDNA library was constructed via suppression subtraction hybridization using human fetal cochlea RNA (16-22 weeks) as the starting material. The procedure was effective as could be shown by the enrichment of cDNAs representing cochlea-specific genes and the subtraction of cDNAs representing house keeping genes. After sequencing, 2616 clones could be analyzed further by BLAST analysis with EST-databases and databases with genomic sequences. Thereby, the majority of the clones could be accurately mapped on the human genome. Based on the BLAST analysis the clones can be grouped in five clusters. (1) Clones that represent a known gene (32 %) including several known deafness genes. (2) Clones that show homology with a known genomic sequence but not with known ESTs (32%). (3) Clones that show homology with known ESTs, but not with known genomic sequences (4%). (4) Clones that show homology with both known genomic and EST sequences. (5) Clones that show neither homology with known genomic sequences nor ESTs (4%). Clones in clusters 2, 4, and 5 represent, at least in part, new genes. To select clones representing genes that are specifically and/or preferentially expressed in the cochlea and thereby representing candidate genes for inherited hearing loss, we performed a semi-quantitative RT-PCR on twelve human fetal tissues. We first studied clones located in known deafness loci for which the causative gene has not yet been identified. Clones located in 9 DFNA loci and 12 DFNB loci were present in the analyzed clone set and a number of interesting clones emerged from this analysis. Now, clones from cluster 2 and 4 are under study. A selection of clones will be studied in more detail by isolating the full-length cDNA sequence and by determining the intron-exon structure. To address involvement of these genes in hearing loss, they will be tested for the presence of mutations in relevant families and/or a patient cohort.

Low frequency of the GJB6 342 Kb deletion in the belgian deaf population. *P. Hilbert¹, W. Wuyts², I. Del Castillo³, L. Mariage¹, N. Peeters², S. Ligny⁴, I. Courtmans⁴, A.F. Fourneau⁵, F. Moreno³, G. Van Camp², L. Van Maldergem¹.*
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Congenital deafness is observed in 1 out of 1000 newborns. Approximately 50 % of these are thought to have a monogenic cause. The majority of individuals with non-syndromic genetic hearing loss show an autosomal recessive inheritance and mutations in GJB2 gene, encoding the gap-junction protein connexin 26, are responsible for about 50% of those cases. Interestingly, different studies have reported that 10 to 40 % of patients present only one mutant GJB2 allele. This proportion, unexpected since far higher than the population carrier frequency (varying from 2 % in the USA to 4 % in Italy), suggested the involvement of a mutation in a non-coding part of the gene or of a digenic effect. Recently, a 342 kb deletion upstream of GJB2, deleting another connexin gene (GJB6) located less than 50kb away, was shown to be associated to deafness both in the homozygous status and in heterozygosity with a GJB2 point mutation in trans. This deletion, called D(GJB6-D13S1830) was found in 22 out of 44 patients (50 %) heterozygous for a single GJB2 mutation from the Spanish population. This proportion raised to 22/33 (65%) if patients clearly not linked to DFNB1 were removed. The aim of our study was to evaluate the importance of the GJB6 in the belgian population. We analyzed 32 deaf individuals who were heterozygous for only one GJB2 mutation. Only 4 of these 32 heterozygous patients (13%) had the DGJB6 deletion. Our study shows that the DGJB6 deletion is significantly less frequent in Belgian GJB2 heterozygotes compared to Spain (2/32 versus 22/44 of the GJB2 heterozygotes, $p=0.001$). Our results indicate that DGJB6 is not sufficient to explain the excess of GJB2 heterozygotes in Belgium, and that additional mechanisms must exist.

A mutation of ESPN causes autosomal recessive nonsyndromic hearing loss, *DFNB36*. S. Naz¹, S. Riazuddin¹, S. Riazuddin³, A.J. Griffith², T.B. Friedman¹, E.R. Wilcox¹. 1) Section on Human Genetics, LMG, NIDCD, Rockville, MD; 2) Section on Gene Structure and Function, LMG, NIDCD, Rockville, MD; 3) National Centre of Excellence in Molecular Biology, Punjab University, Lahore, Pakistan.

In the inner ear, the stereocilia of hair cells contain parallel bundles of cross-linked actin filaments. Espin functions as an actin-bundling protein. A mutation in *Espn* encoding espin causes deafness and vestibular dysfunction in the *jerker* mouse (Zheng et al. 2000, Cell 102:377). We have ascertained a family segregating hereditary hearing loss due to a mutation of *ESPN*. Hearing loss is one of the most prevalent sensory defects in humans. Since 1994, 35 nonsyndromic autosomal recessive deafness loci have been mapped, designated as *DFNB1* to *DFNB35*, and 15 of these genes have been identified. We performed a genome-wide search to identify the gene responsible for hearing loss in a consanguineous family from Pakistan with four affected individuals exhibiting prelingual, sensorineural, profound deafness. A 6 cM region was identical by descent in the affected individuals on chromosome 1p36 between markers *DIS2845-DIS2694*. A two-point lod score of about 4.0 was obtained with the linked markers defining a new recessive deafness location, *DFNB36*. *ESPN* is one of the genes in the 1p36 critical interval and was a candidate for *DFNB36* due to the association of a mutation in the mouse ortholog *Espn* with hearing loss in *jerker* mice. *ESPN* exons were PCR amplified and sequenced. We found a mutation in *ESPN* co-segregating with hearing loss. All affected individuals were homozygous for a four base pair deletion resulting in a frameshift predicted to affect the last thirty amino acids of *ESPN*. The obligate carriers in the family were heterozygous and this mutation was not detected among 150 unaffected control DNA samples from Pakistani individuals. Deletion mutagenesis experiments have suggested that *Espn* contains two actin-binding sites at its carboxy terminus (Bartles et al. 1998, J Cell Biol 143:107). The mutation identified in the affected individuals of the *DFNB36* family affects the second of the two actin binding domains. Our results establish *ESPN* as an essential protein for hearing in humans.

Mapping of a new autosomal recessive nonsyndromic hearing loss locus (DFNB32) to chromosome 1p13.3-22.1. S.

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Approximately 80% of the hereditary hearing loss is nonsyndromic. Isolated deafness forms transmitted in the recessive mode are the most frequent (85%) and the most severe. Thirty-one different DFNB loci have been mapped and 13 of the corresponding genes have been identified. We have ascertained a large consanguineous Tunisian family with congenital profound autosomal recessive deafness. All affected individuals are otherwise healthy, with normal life spans and without dysmorphic or other abnormal findings. Genotype analysis excluded linkage to known recessive deafness loci in this family. Following a genome wide, a linkage was detected only with locus D1S206 on chromosome 1, thereby defining a novel deafness locus, DFNB32. In order to confirm linkage and for fine mapping the genetic interval, 12 individuals belonging to this family were added and 19 microsatellite markers were tested. A maximum two-point lodscore of 4.1 was obtained at marker D1S2896. The interval of DFNB32 locus overlap with DFNA37 locus. In an effort to identify the DFNB32 gene, we have tested the Coll11A1 gene that have been mapped to the DFNB32 interval. This gene is an excellent candidate because : (i) is responsible of two syndromic deafness, Marshall and stickler syndromes (ii) mutations in the homologue mouse mutant produce hearing impairment. The entire coding region of Coll11A1 was screened and no mutation was observed. Towards the identification of the gene, a search of the Human Cochlear cDNA Library and EST Database was done. A gene corresponding to one of the EST found is being screened.

Five novel mutations in the SLC26A4 gene, associated with Pendred Syndrome. *L.H. Hoefsloot, A. Claassen, M. Leferink, I. de Wijs, H. Scheffer.* Dept Human Genetics, University Medical Center, Nijmegen, Netherlands.

Pendred Syndrome is characterized as an autosomal recessive congenital neurosensory deafness, often accompanied by a vestibular function defect, cochlear malformation, and goiter. Mostly patients are euthyroid, with compensated hypothyroidism. Pendred Syndrome is caused by mutations in the SLC26A4 gene.

The most common form of inner ear abnormality, enlarged vestibular aqueduct (EVA), is associated with characteristic clinical findings, including fluctuating and sometimes progressive sensorineural hearing loss and disequilibrium symptoms. Patients with EVA do not fulfill the criteria for Pendred syndrome, but in many cases also mutations in the SLC26A4 gene are found.

Despite its apparent homology to sulphate transporters, the SLC26A4 gene encodes a chloride-iodide transport protein. Iodide is an important anion in the thyroid, and this might explain the thyroid involvement. Its precise function in the mid-ear is uncertain, but given the enlargement of the aqueduct in most cases, a function in the chloride transport has been inferred.

Twenty two patients diagnosed or suspected with Pendred Syndrome or EVS were sent for mutation analysis in the SLC26A4 gene. All 21 exons including the intron-exon boundaries were examined using sequence analysis. In 10 patients two mutations were identified. Of these 20 alleles, 7 alleles (35%) had the L236P mutation and 2 (10%) had the T416P mutation. However, 6 alleles carried novel mutations, including one novel mutation that was encountered twice (M1T), both times in combination with the L236P mutation. The other changes were two missense mutations (S392N, G102R) and two splice site mutations. In one patient only one previously described mutation (V138F) was detected. Clinical data will be matched with the mutations found to search for genotype-phenotype relations.

The vestibular Headbanger mouse mutant with long and circular stereocilia: myosin VIIa as a candidate gene. *R.*

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The ethylnitrosourea (ENU) mutagenesis program is a systematic approach for the identification and characterization of genes involved in hearing loss. The Headbanger *Hdb* mouse mutant is a dominant mutation generated by the ENU mutagenesis program. The behavior of the mouse is suggestive of a vestibular dysfunction. The Headbanger phenotype consists of a circular arrangement of the outer and inner hair cells stereocilia in the cochlea, mainly in the apex, with progressive splaying and fusion of the inner hair cells stereocilia. The hair cells in the utricle are very long, thin and wispy. Seventy-one N2(C3HxC57BL/6) mutant mice were generated using a backcross mapping protocol. Genomic DNA extracted from the tails of these mice was used to construct a low resolution map using microsatellite repeat markers and SNPs in 3 and 5' UTRs, polymorphic between C3H and C57BL/6. A computer software named Pattern was developed to summarize and analyze genotyping results in the format of haplotypes. The chromosomal localization of the mutation was traced to a 1.5cM interval on chromosome 7 that spans over 8 million base pairs. By intercrossing F2(C3HxC57BL/6) mutants, mice homozygous for the mutation were generated. Using a positional-candidate gene approach, an A/T transversion was found in codon 531 of mouse myosin VIIa mRNA causing an Isoleucine to Phenylalanine amino acid substitution. Myosin VIIa protein levels in the cochlea of the mutant mice were assayed by western blot analysis, and showed a down-regulation of protein levels when compared to controls. Immunohistochemical analysis of postnatal day 1 organ of Corti explants did not reveal mislocalization of the protein. It is possible that another ENU induced mutation within the above mentioned linkage interval is responsible for the Headbanger mouse phenotype.

The D(GJB6-D13S1830) deletion with GJB2 mutations in Brazilian families. *E. Sartorato*¹, *C.A. Oliveira*¹, *F. Alexandrino*¹, *A.T. Maciel-Guerra*². 1) Lab de Genetica Humana/CBMEG, Univ Estadual de Campinas, Campinas, SP., Brazil; 2) FCM - Depto Genetica Medica, Univ Estadual de Campinas, Campinas, SP., Brazil.

Mutations in the connexin 26 gene (Cx-26; GJB2) are the most common cause of nonsyndromic hearing loss in many world populations. However, approximately thirty percent of the patients with a Cx26-mutation show only a monoallelic mutation whose pathogenicity has not been proved. We studied 12 patients with Cx-26 mutation in only one allele. In the first family an apparent "normal" allele was segregating with hearing loss. Our index case, a 13-month-old girl, was referred due to bilateral profound sensorineural deafness. She was the first child of a 29-year old woman and her unrelated 29-year-old husband, both of them deaf. Her father, as well as a paternal aunt, had congenital profound sensorineural deafness. In turn, her mother and a maternal aunt had normal speech development in infancy, and hearing difficulties were noted after the age of six years; both of them have now profound sensorineural hearing loss. The paternal and maternal grandparents had normal hearing and were not consanguineous. Both the paternal and the maternal aunts had deaf husbands, and both gave birth to normal hearing boys. We found the deletion D(GJB6-D13S1830) cooperating with the 35delG and V95M mutations in different members of the family. The contribution of the deletion to the phenotype of deafness remains to be explained. We also found this deletion in other family, together with a heterozygous GJB2 mutation. We must be aware to the need of studying D(GJB6-D13S1830), which is now described also in Brazilian families.

High prevalence of W24X mutation in Indian families with Non-Syndromic Deafness. *R. Vijaya, M. Ghosh, M. Kabra, P.S.N. Menon, R.C. Deka**. Genetics Unit, Department of Pediatrics & Department of Otorhinolaryngology*, All India Institute of Medical Sciences, New Delhi, India.

Mutations in the gene GJB2, coding for the gap-junction protein Connexin 26 are responsible for more than 50% cases of prelingual non-syndromic deafness in many Caucasian populations. Among these, the 35delG mutation accounts for up to 85% in Mediterranean population, 70% in United States, and 58% in Israel. The 167delT mutation accounts for 42% of the connexin 26 mutations in Israel and frequent in Ashkenazi Jews while the R134W mutation is common in Africans. Among the Asian countries, 235delC is common in the Japanese population with a frequency of 1-2%. In order to evaluate the extent to which 35delG and three other mutant alleles W24X, W77X and Q124X, contribute to the non-syndromic deafness in Indian population, we screened 329 affected individuals belonging to 110 families from various States of India which included 16 families with sporadic cases. All the families had three or more individuals affected who also had affected relatives. The degree of hearing loss in the affected individuals was either severe and/or profound but intrafamilial variability was notable. Genomic DNA was extracted and the four mutations were detected by allele-specific PCR. Out of 110 families, 36 families (32.7%) were positive for these mutations and a total of 175 mutant alleles were identified. Amongst the 36 families, 26 families (72.2%) were homozygous and 5 families were heterozygous for W24X mutation. Three families were biallelic, W24X/W77X in two families and W77X/35delG in one family. Two sporadic cases were homozygous for Q124X. Therefore mutations in GJB2 gene represent a major cause of recessively inherited non-syndromic deafness in Indian population. It appears that 35delG may not be a common mutant allele as observed in other populations but W24X was identified frequently. This demands a need to evolve a strategy for screening for the mutations in the GJB2 gene in the Indian subcontinent. Availability of a simple DNA based diagnosis for the detection of W24X is indispensable and molecular diagnosis becomes an integral part of genetic counselling for non-syndromic deafness.

Genotype-phenotype correlation and expression analysis of the WFS1-gene responsible for nonsyndromic hearing impairment and Wolfram syndrome. *G. Van Camp¹, K. Cryns¹, S. Thys¹, L. Van Laer¹, M. Pfister², K. Flothman¹, H. Kremer³, W. Reardon⁴, R.J.H Smith⁵.* 1) Department of Medical Genetics, University of Antwerp, Belgium; 2) Department of Otolaryngology, University of Tübingen, Germany; 3) Departement of Otorhinolaryngology, University Medical Centre St Radboud, Nijmegen, The Netherlands; 4) National Centre for Medical Genetics, Our Lady's Hospital for Sick Children, Crumlin, Dublin, Ireland; 5) Molecular Otolaryngology Research Laboratories, Department of Otolaryngology, University of Iowa, Iowa City.

The WFS1 gene is responsible for Wolfram syndrome, also called DIDMOAD (Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy and Deafness), which is an autosomal recessive disease. In addition, heterozygous mutations have been found in families with fully penetrant autosomal dominant low frequency sensorineural hearing impairment (ADLFHI). This is remarkable, as the hearing impairment in DIDMOAD shows reduced penetrance and mainly affects the high frequencies. We have completed mutation screening of WFS1 in 8 autosomal dominant families and 10 sporadic cases in which affected persons had LFHI. We identified 7 missense mutations and a single amino acid deletion affecting conserved amino acids in 6 families and one sporadic case. The success rate in ADLFHI is high, but the low success rate in simplex cases suggests that there are environmental causes of LFHI or unknown autosomal recessive genes. Among the 10 WFS1-mutations reported in LFHI, none is expected to lead to premature protein truncation and 9 cluster in the C-terminal protein domain. We compared these characteristics to those of the mutations causing Wolfram syndrome, and found some remarkable differences. In Wolfram syndrome, 64% of the mutations are inactivating, and the mutations are evenly spread over the complete protein. We have performed expression analysis of the WFS1 gene in the inner ear in mice and rats at different stages of development using immunohistochemistry and in situ hybridization. Both techniques showed compatible results, and indicated clear expression in different cells of the inner ear at different stages of development.

Secretion and subcellular localization of wild-type cochlin, and of mutants causing the sensorineural deafness and vestibular disorder, DFNA9. *N.G. Robertson¹, V. Patriub¹, S.A. Hamaker¹, J.C. Aster^{1,3}, C.C. Morton^{1,2,3}.* 1) Depts. Pathology; 2) Ob/Gyn, Brigham & Women's Hosp; 3) Harvard Medical School, Boston, MA.

Five missense mutations in the *COCH* gene have been identified in the FCH/LCCL domain of its encoded protein, cochlin, and are pathogenic for the autosomal dominant hearing loss and vestibular dysfunction, DFNA9. This domain was shown to have a novel fold; *in vitro* expression of this domain with 3 of 4 DFNA9 mutations results in its misfolding. Misfolded proteins are known to accumulate and become prone to degradation, or to aggregate and cause toxicity. The observation of eosinophilic deposits in DFNA9-affected inner ear structures may also indicate aberrant folding, secretion, or solubility of the mutated protein. To study the biological consequences of misfolding in cochlin, we performed transient transfections of wild-type and mutant cochlin constructs into mammalian cells in culture. The entire coding region of *COCH* (~1.65 kb, 550 aa) was cloned into a eukaryotic expression vector with C-terminal HA tags. Three missense mutations were introduced separately into the construct and transfected into 293T- HEK, COS-7, and 3T3 cell lines. Immunocytochemistry revealed concentrated localization of cochlin in perinuclear structures consistent with the Golgi apparatus and endoplasmic reticulum for both wild-type and mutant cochlin. Western blots of total protein lysates from transfected cells and of the secreted media immunoprecipitated with anti-HA showed equal amounts of normal and mutant cochlin in the cellular fraction and in the media from transfected cells. These results indicate that wild-type and mutant cochlin expressed in mammalian cells are secreted in a similar fashion via the Golgi/ER secretory pathway and that the presence of these mutations does not appear to interfere with secretion or lead to intracellular retention and/or degradation of the protein. Alternative mechanisms of pathology caused by these missense mutations may be the loss of function of the FCH/LCCL domain or lack of proper folding and binding of this domain to other components in the extracellular matrix of the inner ear.

Mutations in myosin IIIA cause nonsyndromic progressive hearing loss DFNB30. *V. Walsh¹, T. Walsh¹, S. Vreugde², R. Hertzano², H. Shahin^{2,3}, S. Haika², M.K. Lee¹, M. Kanaan³, M.C. King¹, K.B. Avraham².* 1) Medicine and Genome Sciences, Univ. of Washington, Seattle, WA; 2) Department of Human Genetics and Molecular Medicine, Sackler School of Medicine, Tel Aviv Univ., Israel; 3) Department of Life Sciences, Bethlehem Univ., Bethlehem, Palestinian Authority.

The DFNB30 locus is represented by an extended Israeli family originating in Mosul, Iraq. The hearing loss in this family is progressive, beginning in the second or third decade of life. Until now, all families reported with progressive hearing loss showed a dominant pattern of inheritance. A genome scan revealed age-dependent recessive inheritance, although homozygosity was restricted to only a part of the critical region. Therefore it was not surprising to discover several mutations in the MYO3A gene, corresponding to the haplotypes segregating in the family. The hearing loss is caused by three different loss-of-function mutations in myosin IIIA: a nonsense mutation and two splice site mutations. Most interesting, individuals who were homozygous for the nonsense mutation had an earlier onset of hearing loss and their hearing loss is more severe than that of individuals that are heterozygous for the nonsense and either of the splice mutations. Class III myosins are hybrid motor-signaling molecules, with an N-terminal kinase domain, highly conserved head and neck domains, and a class III-specific tail domain. Expression of mammalian myosin IIIA is highly restricted, with the strongest expression in retina and cochlea. Furthermore, recessive null mutations in the *Drosophila* homolog of MYO3A (NINAC) delay termination of the photoreceptor response and lead to progressive retinal degeneration. Although hearing impaired individuals have never complained of visual problems, detailed ophthalmological tests will determine whether the retina is affected, as another myosin is involved in both deafness and retinitis pigmentosa. In *Drosophila* rhabdomeres, NINAC interacts with actin filaments and with a PDZ scaffolding protein to organize the phototransduction machinery into a signaling complex. We will present data from our yeast 2-hybrid screen in our attempts to identify the mammalian myosin III interactors.

The spectrum of GJB2 and mtDNA mutations in prelingual nonsyndromic deafness in Greece. A. Pampanos¹, M. Grigoriadou¹, T. Iliades², N. Voyiatzis², J. Economides³, P. Leotsakos³, P. Neou⁴, M. Tsakanikos⁴, T. Antoniadi⁵, A. Hatzaki⁵, I. Konstantopoulou⁶, D. Yannoukakos⁶, J. Gyftodimou¹, A. Skevas⁷, M.B. Petersen¹. 1) Inst. of Child Health, Athens, Greece; 2) Aristotle Univ. of Thessaloniki, Greece; 3) "Aghia Sophia" Children's Hosp., Athens, Greece; 4) "P. & A. Kyriakou" Children's Hosp., Athens, Greece; 5) "MITERA" Maternity Center, Maroussi, Greece; 6) N.C.S.R. "Demokritos", Athens, Greece; 7) Univ. of Ioannina, Greece.

Mutations in the GJB2 gene encoding connexin 26 have been shown as a major contributor to prelingual nonsyndromic deafness, with one specific mutation (35delG) accounting for the majority of mutations detected in the GJB2 gene in Caucasian populations. Several mtDNA mutations have also been reported as causes of nonsyndromic childhood hearing impairment, but a more precise estimate of the prevalence of these mutations has only been analyzed in a few population-based studies. In a collaboration with the major referral centers for childhood deafness in Greece, patients were examined by ARMS-PCR for the detection of the 35delG mutation, direct genomic sequencing of the GJB2 gene, and PCR-RFLP for the detection of mtDNA mutations A1555G, A7445G, and 7472insC. A total of 232 patients with prelingual sensorineural deafness were studied, where syndromic forms and environmental causes of deafness had been excluded. Biallelic GJB2 mutations were found in 78 patients (33.6%): 68 35delG/35delG homozygotes and 10 compound heterozygotes with the 35delG mutation and another mutation (L90P 3 alleles, W24X 2 alleles, R184P 2 alleles, delE120 1 allele, 291insA 1 allele, and 88delA 1 allele). The homoplasmic A1555G mutation was detected in two cases (one sporadic and one familial) and the A7445G mutation was found in one sporadic case of mild hearing impairment. We conclude that GJB2 mutations are responsible for a large proportion of prelingual nonsyndromic deafness in the Greek population. Mutations in the mtDNA are responsible for only a small fraction of prelingual deafness, but the identification of an mtDNA mutation is vital for genetic counseling.

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Analysis of Connexin 26 mutations in a population of deaf patients. *V.A. Stevenson¹, M. Ito¹, J.M. Milunsky^{1,2}*. 1) Center for Human Genetics, Boston University School of Medicine, Boston, MA; 2) Department of Pediatrics, Boston University School of Medicine, Boston, MA.

The most common genetic cause of non-syndromic hearing loss are mutations in the Connexin 26 gene. At the Center for Human Genetics, samples sent for Connexin 26 mutation analysis are usually first screened for the common mutations 35delG and 167delT [Ashkenazi Jewish population] and then sequenced if requested. Between January 2001 and May 2002, we identified 14 35delG homozygotes, 3 167delT homozygotes, and 1 35delG/167delT compound heterozygote. In addition, sequence analysis identified 34 other affected alleles. Fifteen individuals were compound heterozygous for 35delG and another mutation. Two individuals were compound heterozygous for 167delT and another mutation. Eleven individuals were either heterozygous (5), homozygous (2), or compound heterozygous (4) for various other Connexin 26 mutations. In addition, we report 5 new mutations in the Connexin 26 gene: F191S, E129X, W3C, L6W, and del151TTinsC. These mutations were all identified in patients with at least one other mutation in the Connexin 26 gene, and thus likely represent novel disease-causing mutations. In conclusion, screening only for the common mutations 35delG and 167delT could fail to diagnose up to one quarter of affected individuals. A large portion of individuals in whom Connexin 26 mutations other than the common mutations were found were of non-Caucasian ancestry. Therefore, we recommend full Connexin 26 sequencing in those affected individuals of non-Caucasian ancestry.

Auditory Neuropathy in a family with Mohr-Tranebjaerg Syndrome due to a nonsense mutation in TIMM8a.

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Mohr-Tranebjaerg Syndrome (MTS) is characterized by early-onset post-lingual deafness and late-onset dystonia or ataxia, progressive visual deterioration, paranoid psychotic features and mental deterioration. Mutations in TIMM8a have been shown to cause MTS by not allowing the import of the hTim23 protein into the mitochondria. TIMM8a is a nuclear-coded gene located on Xq22. A family with three affected males in two generations have been found to have a nonsense mutation, 135 C>T (Q34X), in exon 1 of the TIMM8a gene. These males have many of the classical features associated with MTS, but in addition the hearing loss has been determined in the youngest affected family member to be an auditory neuropathy. Auditory neuropathy is a type of hearing loss characterized by absent or abnormal auditory brainstem response with normal outer hair cell function. It was predicted that an auditory neuropathy phenotype would be associated with MTS by Merchant et al. (*Otol Neurotol.* 2001 Jul;22(4):506-11) who found near complete loss of spiral ganglion cells and preserved organ of Corti in the temporal bone of an MTS patient with the 151delT mutation in TIMM8a. The case presented here is the first where outer hair cell function was determined clinically in an MTS patient. This patient had an unsuccessful trial with hearing aids and has since stopped wearing them. While cochlear implants have been shown to be beneficial in some cases of auditory neuropathy, the family has been informed of the studies by Merchant, et al. and been advised that a cochlear implant might not work due to the progressive loss of the spiral ganglion. This family verifies the prediction that MTS is associated with an auditory neuropathy and demonstrates the importance of a genetic diagnosis in deciding the type of therapy for hearing disorders.

Total absence of the alpha2(I)Collagen chain due to a homozygous COL1A2 mutation in a patient with an EDS-like phenotype. A. De Paepe¹, S. Symoens¹, L. Nunes², S. De Almeida², K. Wettinck¹, R. Dekens¹, F. Malfait¹, P.J. Coucke¹. 1) Medical Genetics, 0K5, Univ Hosp Gent, Ghent, Belgium; 2) Medical genetic service, Hospital Dona Estefania, Lisboa, Portugal.

We report a homozygous mutation in Type I Collagen (COL1A2) in a 6 year old Portuguese boy from a consanguineous marriage presenting an Ehlers-Danlos (EDS) phenotype with generalized joint hyperlaxity, hypotonia, skin hyperextensibility, external rotation of the hands and long, thin fingers. Biochemical analysis on skinfibroblasts showed the presence of proalpha1(I) and alpha1(I) chains but no evidence of proalpha2(I) or alpha2(I) chains neither in the medium nor in the cell layer. Mutation analysis of the genomic DNA revealed a homozygous one basepair insertion in exon 7 of the COL1A2 gene (COL1A2-292insC). The resulting frameshift introduces a premature termination codon in the next aminoacid position. This mutation was also identified in the heterozygous form in both parents who are apparently healthy. Until now, only 4 patients with a total alpha2(I) collagen chain deficiency have been described. They show however markedly different phenotypes: one presented severe Osteogenesis Imperfecta (OI) type III, whereas the 3 other patients presented a mild, EDS-like or EDS/OI phenotype. Although the clinical study of our family is still in progress, the phenotype is mild and resembles that of the 3 EDS patients. It is unclear how an apparent total lack of the alpha2(I) chains can produce such differences in phenotypic severity. In the patient with OI Type III and the one with OI/EDS phenotype the causal COL1A2 mutation was identified as a 4 bp deletion and a splice site mutation, respectively. In the latter patient, it was assumed that minute amounts of normal alpha2(I) chains are still produced, which ameliorate the clinical phenotype from a severe OI to a much milder EDS/OI phenotype. Here, the presence of a homozygous stopcodon in exon 7 (resulting from a frameshift), renders this mechanism unlikely. Other genetic factors must be responsible to explain the predominant phenotypic expression in the soft tissues and the minimal degree of bone fragility in the present case.

Development of a human muscle expression data warehouse. *M.A. Bakay, J. Chen, E.P. Hoffman.* Research Center for Genetic Medicine, Childrens National Medical Center, Washington DC.

Data generated on DNA sequence, mRNA ESTs, and emerging SNPs is now integrated into genome browsers on the relatively static DNA code. Transcription and proteome profiles have many more variables that make integrated databases more challenging, and we have shown that the associated noise from experimental variability, tissue heterogeneity, and inter-patient SNP noise are considerable factors to consider (Bakay et al. 2002). To begin to develop publicly accessible data warehouses on human tissues, we have focused on muscle in both health and disease, using whole genome-anchored Affymetrix U133 arrays, and Oracle web interfaces with queries enabled across all profiles, and across species. Here we an initial installment of 25 patient muscle biopsies, including Duchenne muscular dystrophy, Becker muscular dystrophy, dysferlin deficiency (LGMD2B), and normal controls. For the sake of this presentation, we focus on changes of the dystrophin mRNA as a function of diagnosis. We found that dystrophin mRNA was statistically significantly reduced relative to controls, consistent with nonsense-mediated RNA decay and frame shift mutations. However, Becker dystrophy patients show an increase in dystrophin mRNA relative to controls, consistent with compensatory up-regulation due to the semi-functional protein. Dysferlin deficient patients, while showing secondary reductions of dystrophin by immunocytochemistry, showed normal levels of dystrophin mRNA. We then tested whether the large-scale profiling of biopsies would permit the delineation of expression fingerprints that are diagnostic of different underlying defects. We compared DMD-vs-Dysferlin and BMD-vs-Dysferlin deficient samples. We have found 149 and 82 genes survived cut-off $p < 0.05$ and Fold Change > 2 for Dysferlin-vs-DMD and Dysferlin-vs-BMD, respectively. Dysferlin gene had higher expression in both DMD and BMD groups with FC = 2.1 and $p = 0.01$ (DMD) and 0.004 (BMD). Dystrophin genes was significantly down regulated in DMD-vs-Dysferlin groups with $p = 0.004$ and FC = 5. Our data suggests that diagnostic profiles can be developed, and signature genes reflective of each disease can be identified.

The human cyclic nucleotides phosphodiesterase (PDE) Prune protein: a dual cellular compartment localization and functional properties. A. Dangelo¹, A. D'Andre¹, P. Carotenuto¹, V. Aglio¹, L. Garzia¹, G. Arrigoni², A. Ballabio¹, M. Zollo¹. 1) TIGEM, TIGEM, Naples, ITALY; 2) HSR San Raffaele, Laboratorio di Anatomia Patologica, Milan, ITALY.

The human prune homologue gene is located on 1q21.3 epidermal cluster region. The gene is frequently amplified in sarcoma and breast cancer. A model of negative regulation of prune versus nm23-H1 anti-metastasis protein has been postulated. On the basis of sequence alignment we found a newly identified phosphodiesterase (PDE) PDE11A containing a catalytic site motif equally present in the human prune protein. To investigate prune activity a scintillation proximity assay was performed on the purified histidine-tagged prune protein produced by the Baculovirus expression system. Prune is functioning as a cyclic nucleotides phosphodiesterase preferentially on cAMP substrate. In progress are the analysis with different inhibitors of prune PDE activity since phosphodiesterase inhibitors are considered as potential anti-inflammatory and anti-proliferative agents in human skin. The human PRUNE gene is expressed in the basal and granular layers of human skin and map in the epidermis cluster locus where two skin multigenic disorders have been identified so far (Atopic Dermatitis OMIM # 603165 and Psoriasis OMIM# 177900). We have cloned the human PRUNE cDNA under the control of the loricrin promoter, to drive the expression in both the undifferentiated and differentiated skin layers. Four founders lines have been produced. Prune protein is predominantly a cytoplasmic protein. By direct immunofluorescence experiments we have investigating prune and nm23 protein localization in SK-N-SH, SK-N-BE, SH-5YSY and IMR-32 Neuroblastoma-derived cell-lines. A predominant prune nuclear localization was observed for the first time. Functional studies of the role of prune in the nucleus compartment will be presented.

Program Nr: 2010 from 2002 ASHG Annual Meeting

Enzyme activity of GTP cyclohydrolase I mutations and their relevance to dopamine insufficiency. *S.M. Hague, C. Evey, M. Baptista, M. Cookson, J. Hardy, A. Singleton.* Neurogenetics, NIA, Bethesda, MD.

Mutations in GTP cyclohydrolase I (GCH-1) the rate limiting enzyme in tetrahydrobiopterin biosynthesis are associated with a number of diseases of dopamine insufficiency, including Dopa-responsive dystonia and hyperphenylalanemia (HPA). Currently, there are 60 reported mutations in GCH-1. Mutations have been shown to decrease the level of enzyme activity with a concomitant reduction in dopamine synthesis. Previously, we have also demonstrated GTP cyclohydrolase mutations in 3 of 180 cases of parkinsonism. In the three families studied a Pro23Leu transition was identified and in one of these an additional Pro69Leu was observed in cis with the Pro23Leu mutation. We postulate in the families examined these observed mutations may reduce the level of tetrahydrobiopterin synthesis and ultimately the level of dopamine, contributing to the observed parkinsons disease. To test this hypothesis we have assayed the level of GCH-1 using a formate release assay in a number of cell lines from PD patients and from additional controls. We report the results of this assay herein and discuss the implications of this finding in parkinsons disease.

Accumulation of deoxyuridine due to primary thymidine phosphorylase deficiency. *R. Marti, Y. Nishigaki, M. Hirano.* Neurology, Columbia University, New York, NY.

Background: Mitochondrial NeuroGastroIntestinal Encephalomyopathy (MNGIE) is an autosomal recessive disorder caused by loss-of-function mutations in the gene encoding thymidine phosphorylase (TP). In MNGIE patients, depletion, multiple deletions and point mutations of mitochondrial DNA (mtDNA) have been identified. We have previously described alteration of thymidine metabolism in these patients, with high circulating concentrations of thymidine. In the present work, we screened for alterations in the levels of other circulating nucleosides in the plasma of MNGIE patients, that could contribute to the pathomechanism of this disease.

Material and Methods: Reversed phase HPLC, with elution in gradient and with UV detection was used to quantify the circulating concentration of nucleosides. Plasma samples from 22 patients and 20 healthy controls were analyzed.

Results: Similar to previous results, we identified increased levels of thymidine in MNGIE patients (8.1 ± 3.2 mM; range 3.9 - 17.5) compared to controls (<0.05 mM). In addition, deoxyuridine (dUrd) was present in the plasma of all analyzed MNGIE patients (13.2 ± 3.6 mM; range 5.5 - 21.8). No detectable dUrd was found in any of the analyzed controls (<0.05 mM). Concentrations of dUrd in the patients were slightly higher than the corresponding levels of thymidine.

Conclusion and Hypotheses: Loss of function of TP in MNGIE patients leads to extracellular accumulation of dUrd, in addition to the previously described increases of thymidine. Extracellular accumulation of dUrd in MNGIE patients could result in increases of the normally low intracellular levels of deoxyUTP, leading to incorporation of uracil into mtDNA. Uracil misincorporation may contribute to the mtDNA alterations observed in this disease.

Affymetix Genechip expression profiling of CLN6-deficient late infantile NCL. *R.N. Boustany¹, C.A.F. Teixeira^{1,2}, S. Lin³, M.C.S Miranda^{2,4}, M.G.R. Ribeiro^{2,4}.* 1) Div Pediatric Neurology, Duke Univ Medical Ctr, Durham, NC; 2) Unidade de Neurobiologia Genetica do Instituto de Biologia Molecular e Celular da Universidade do Porto, Portugal; 3) Duke Bioinformatics Shared Resource, Duke Univ Medical Ctr, Durham, NC; 4) Unidade de Enzimologia do Instituto de Genetica Medica do Porto, Portugal.

The neuronal ceroid lipofuscinoses (NCLs) are a group of autosomal recessive neurodegenerative diseases characterized by cognitive and motor decline, blindness and seizures and by progressive neuronal loss in the brain and retina. Six NCL genes have been identified, the most recent of which is CLN6, a novel transmembrane protein of unknown function. In this study Affymetrix Genechip analyses were performed in cultured fibroblasts from normal controls and a number of NCL types. The CLN6-deficient cases included 3 Portuguese patients homozygous for the I154del common Portuguese mutation and 1 Costa Rican patient homozygous for the common Costa Rican c.214GT mutation. The CLN6-deficient profiles were distinct from all other NCL variants analyzed suggesting a unique pathobiology for this variant, and a possible role for the use of genechip technology in the identification of new disease variants. We applied a threshold that identified genes with 2-fold change or more in either direction in comparison with the control. This analysis generated a group of 16 up and down-regulated genes that provide clues as to the function of this novel gene as well as some insight into the cell biology of this NCL variant. The function and interrelationship of these sixteen genes will be discussed.

***MutationView* : A Graphical On-line Database System for Mutations and Polymorphisms in Human Disease**

Genes. *S. Minoshima*¹, *M. Ohtsubo*¹, *S. Mitsuyama*¹, *S. Ohno*¹, *T. Kawamura*¹, *S. Ito*², *F. Ito*³, *N. Shimizu*¹. 1) Dept Molec Biol, Keio Univ Sch Med, Tokyo, Japan; 2) Dept Environ Health Sci, Setsunan Univ, Hirakata, Japan; 3) Dept Biochem, Setsunan Univ, Hirakata, Japan.

More than 1100 disease-causing genes have been reported. Databases for mutations in these disease-causing genes are indispensable for the diagnostics, therapeutics and basic research of the diseases. At present, mutation data are maintained as individual Locus-specific Databases (LSDBs) only for selected numbers of diseases and therefore more comprehensive database system with common graphical user-interface is required. We have established the *MutationView* as an integrated graphical mutation database. Currently, the *MutationView* has collected 8176 entries of mutations/polymorphisms from 1497 literatures, dealing with 200 genes involved in 190 distinct diseases. The characteristic features of the *MutationView* are as follows:

(1) Several ways are available to have an access to the gene of interest through the chromosomal map of the gene or disease, anatomical charts of disease-associated organ or tissue, and schematic figures of causative gene products and related proteins.

(2) Various data display and analysis functions are available: Genomic/cDNA structure of normal gene, functional domain structure of protein, zooming-in and -out of the nucleotide and amino acid sequences, plotting mutations with the histogram of case number, changes in the nucleotide sequence and restriction sites, classification based on mutation type, dominant/recessive and symptom, experimental information such as PCR primers and reaction conditions.

(3) Mutation data can be placed in any web server and therefore *MutationView* system is ideal to link global mutation databases managed by LSDB curators.

The user ID and password are issued upon formal applications through the URL <http://mutview.dmb.med.keio.ac.jp>. Computer demonstration will be performed at the meeting.

Sequencing of Candidate Genes for Non-Syndromic Cleft Lip and Palate Finds Rare Etiologic Variants. *C.J. Nishimura, J. Harrington, B. Ludwig, J. Allaman, A. Vieira, P. Jezewski, R. Schultz, S. Kondo, J. Avila, A. Warrington, Y. Watanabe, J.C. Murray.* Pediatrics, The University of Iowa, Iowa City, IA.

Non-syndromic cleft lip and palate occurs in about 1 in 1000 live births. We are performing a comprehensive sequencing project to identify etiologic mutations and SNPs in candidate genes for cleft lip and palate. Genes included in this screening project: LHX8, MSX1, P63, PVRL1, PVRL2, SKI1, TGFA, TGFB1, TGFB3, and IRF6. The samples screened are from 100 each of non-syndromic cleft lip and palate cases from Iowa and the Philippines. Primers were developed flanking the coding regions and splice sites for each gene. Sequencing was performed bidirectionally and variants were detected using Polyphred analysis. Variants are confirmed by re-sequencing of the affected proband and by sequencing other family members and controls. Polymorphic variants are being used for transmission disequilibrium studies. Variants in LHX8, MSX1, and SKI1 are being examined for a possible etiologic cause for non-syndromic cleft lip and palate. A variant in exon 7 of SKI1 creates a Proline to Leucine change in a proband with unilateral cleft lip and palate born to unaffected parents, neither of which has the variant. In exon 5 of LHX8, an affected proband, an affected sibling and their unaffected mother have a variant, which changes a Glutamic acid to Alanine. Several variants in MSX1 affect highly conserved amino acid residues. In aggregate these results support a role for rare variants in some cases of non-syndromic clefting and support the polygenic model of etiology.

Skeletal defects and male infertility locus on mouse chromosome 9. K.B. Cha¹, I.J. Karolyi¹, A. Hunt¹, A.M. Wenglikowski¹, J.E. Wilkinson¹, D. Dolan², G. Dootz², A.A. Finnegan³, L.D. Siracusa³, S.A. Camper¹. 1) Human Genetics, Univ. of Michigan Medical School, Ann Arbor, MI; 2) Otolaryngology, Univ. of Michigan Medical School, Ann Arbor, MI; 3) Microbiology and Immunology, Kimmel Cancer Center, Jefferson Medical College, Philadelphia, PA.

Skeletal disorders constitute a diverse group of genetic diseases. Over 200 forms of osteochondrodysplasias have been described. Some have been mapped genetically and are understood at the molecular level, but many forms have unknown etiology. Mouse mutants have contributed significantly to our understanding of skeletal development and homeostasis, and they play an important role in discovering critical genes that would not be predicted based on our current knowledge of skeletal development.

We discovered a spontaneous mutation resulting in growth insufficiency and named it chagun (*cha*), a Korean word for small. Mutant males are sterile. The growth impairment, which follows an autosomal recessive inheritance pattern, is detectable by weaning, and adult mutants weigh approximately 75% of their normal littermates. Skeletal abnormalities include shortened skull, shortened limbs, and enlarged joints. The number of vertebrae and ribs is normal. These results suggest that the growth insufficiency is due to a developmental defect of bone or cartilage. Histological analysis of the femur demonstrates loss of structural integrity of the growth plate and a reduction in trabecular bone in dwarf mice.

The mutant karyotype is normal. To map the mutant locus, mutant females were crossed to *Mus castaneus* males. Intercross progeny demonstrate fully penetrant growth insufficiency and male infertility. A genome scan with polymorphic markers revealed association of the phenotype with distal chromosome 9, which exhibits synteny homology with segments of human chromosomes 3 and 15. Although the parathyroid hormone receptor maps within this region, *Pthr* has been excluded genetically as the cause of growth insufficiency in chagun mice. Thus, identification of the mutant gene in this new mutant mouse may reveal a novel cause of skeletal dysplasia.

Exclusion of the candidate gene matrilin-1 in a cohort of 22 multiple epiphyseal dysplasia (MED) patients. *S.M. Huguenin*^{1,2}, *D.H. Cohn*^{2,3}. 1) UCLA Intercampus Medical Genetics Training Program; 2) Medical Genetics, Department of Pediatrics, Cedars-Sinai Medical Center, Los Angeles, CA; 3) Departments of Human Genetics and Pediatrics, UCLA School of Medicine, Los Angeles, CA.

Multiple epiphyseal dysplasia (MED) is a phenotypically and genetically heterogenous osteochondrodysplasia. Clinically, MED varies from the mild Ribbing type to the severe Fairbank type, with common features including delayed epiphyseal ossification, mild short stature, and joint pain. Six different MED loci have been identified; the genes encoding cartilage oligomeric matrix protein (*COMP*), the three chains of type IX collagen (*COL9A1*, *COL9A2*, *COL9A3*), and matrilin-3 (*MATN3*) in the autosomal dominant forms of MED, and the diastrophic dysplasia sulfate transporter (*DTDST*) gene in an autosomal recessive MED phenotype. However, the known loci only account for approximately 50% of MED patients. The matrilins are a four-member family of non-collagenous oligomeric extracellular matrix proteins, each containing von Willebrand factor A domain(s), EGF-like repeat(s), and a carboxyl terminus coiled-coil oligomerization motif. The tissue expression patterns of *MATN1* and *MATN3* are specific to cartilaginous tissue of the growing skeleton, whereas *MATN2* and *MATN4* have a broader tissue distribution and are mainly expressed in non-skeletal tissues. Furthermore, the *MATN1* and *MATN3* proteins often co-localize and are known to co-assemble into either homo- or hetero-oligomers. The identification of mutations in *MATN3* in two MED families suggested the possibility that mutations in *MATN1* could produce MED. To test this hypothesis, the sequences of the eight exons, including exon/intron boundaries, of *MATN1* were determined in genomic DNA from 22 MED patients in which *COMP*, *COL9A1*, *COL9A2*, *COL9A3*, and *DTDST* loci were excluded. No mutations were identified, excluding structural mutations in *MATN1* as the cause of MED in this cohort. While regulatory mutations cannot be excluded, lack of an overt skeletal phenotype in the *Matn1* knockout mouse suggests that such mutations might not be expected. Our data suggest that if mutations in *MATN1* can cause MED, such defects are rare.

Screening of patients with trigonocephaly with markers from the 9p and 11q regions. *F.S. Jehee¹, D. Johnson⁴, L. Alonso³, K. Abe¹, N. Alonso², H. Matushita², C. Kim², S. Cavaleiro³, S.A. Wall⁴, A.O.M. Wilkie⁴, M.R. Passos-Bueno¹.*
1) CEGH, IBUSP, Brazil; 2) FMUSP, Brazil; 3) UNIFESP, Brazil; 4) Craniofacial Unit, Oxford, UK.

Trigonocephaly is caused by metopic craniosynostosis and can occur as an isolated clinical feature or as part of a syndrome. Deletions in two chromosomal regions, del(9p22-24) and del(11q23-24), are associated with syndromic phenotypes which include almost invariably trigonocephaly. Therefore, there might be genes at 9p and 11q that cause the premature fusion of the metopic suture when in hemizygoty. We are screening patients for hemizygoty in the regions above mentioned to: i) verify if isolated trigonocephaly is caused by microdeletions, ii) reduce the candidate regions for these genes, which currently encompass approximately 10 cM. A cohort of 46 patients with either isolated or syndromic trigonocephaly was studied. Large deletions were found in 6 syndromic cases: one del11q and five del9p. These findings did not reduce the critical regions. The origin of the deletion could be determined in 5 cases, 4 being paternal: three del9p and the del11q and one maternal: del9p. Karyotype analysis was performed in the parents of 5 cases and revealed that two del9p originated by balanced translocations while the other two del9p and the del11q are de novo deletions. No evidence of microdeletions was found in the other 40 patients. This could suggest that a) microdeletions in these regions are quite rare, b) there are false negative results because of non-informative markers and c) the metopic synostosis is caused by mutations within a gene in the region rather than by a microdeletion. We are also screening our patients for mutations in candidate genes within the critical regions. SSCP analysis was carried out for the coding region of the CER1 gene, an antagonist of BMP4, located at 9p23. We identified a novel SNP (CG at position c195), which does not seem to be associated with the trigonocephaly in our patients since its frequency (40%) is the same as in a control sample. No other mutation was detected indicating that CER1 may not be involved in the etiology of this malformation. FAPESP, PRONEX, HHMI.

Osteoporosis Pseudoglioma Syndrome Caused By A Novel, Homozygous, 2-Base Pair Deletion In The LRP5

Gene. *S. Mumm*^{1,2}, *D. Wenkert*², *J. Hagen*¹, *M.P. Whyte*^{1,2}. 1) Div Bone & Mineral Diseases, Washington Univ Sch Medicine, St. Louis, MO; 2) Center for Metabolic Bone Dis and Mol Res, Shriners Hospital for Children, St. Louis, MO.

Osteoporosis pseudoglioma syndrome (OPPG) is an autosomal recessive disorder caused by inactivating mutations in the gene which encodes the low density, lipoprotein receptor-related protein 5 (LRP5). Clinical features include early-onset blindness (described as retinal dysplasia within the first few months of life, or phthisis bulbi with calcifications and cataract formation diagnosed after 1 year of age), micro-ophthalmia, osteoporosis leading to fractures, wormian bones, hypotonia, and developmental delay. A 1-year-old Caucasian girl of aunt/nephew parentage was found by routine eye exam at 4 days-of-age to have no red reflex. Bilateral retina detachment with persistent fetal retinal vasculature were unexplained. Ophthalmologic surgery preserved light/dark perception. At age 11 months, bilateral distal femur fractures occurred during a fall. She was osteopenic. Our assessment showed microcephaly, micro-ophthalmia, hypotonia, and hyperextensibility of all joints. At age 12 months, after she came out of a spica cast, compression fractures of T8 were noted. At 13 months compression fractures of T10-T12, L1, L2, and L4 were also noted. Mutation analysis of LRP5, including sequencing of exons and intron/exon boundaries, revealed a homozygous 2-base pair deletion (3194-3195delAC) in exon 14. This causes a frame shift at amino acid 1065 (D1065fs) and addition of 71 missense amino acids. OPPG reveals that LRP5 acts significantly in bone accrual. In 2002, a single base pair change in exon 3 of LRP5 was shown to cause a benign phenotype, autosomal dominant high-bone mass trait. Deactivating mutations in LRP5 cause OPPG syndrome with 6 homozygous disease-causing frame shift and nonsense mutations in LRP5, as well as 6 heterozygous putative disease causing missense mutations. These mutations were found in a variety of exons but not exon 14. Our patient has a novel, homozygous, 2-base pair deletion in yet another region of LRP5 which is, therefore, important for the normal functioning of the gene product.

Characterization of Peripheral Leukocytes in PAPA Syndrome, an Autoinflammatory Disease. *C.A. Wise¹, D. Zhang¹, H. Liu¹, E.A. Arce², V.M. Pascual².* 1) Seay Research Ctr, Texas Scottish Rite Hosp, Dallas, TX; 2) UT Southwestern Medical School and Baylor Institute for Immunology Research, Dallas, TX.

PAPA (pyogenic arthritis, pyoderma gangrenosum, and acne) syndrome, is an inherited disorder marked by recurring, destructive, early-onset, arthritis. Inflammatory flares lead to massive accumulation of sterile neutrophils within joints and muscle, variously accompanied by skin ulcerations and cystic acne. These clinical features resemble other more common disorders including inflammatory bowel disease and rheumatoid arthritis, suggesting etiological overlap. To reconstruct the pathogenesis of PAPA syndrome we recently identified the responsible gene, PSTPIP1, and demonstrated that disease-causing missense mutations compromise binding to PEST-type protein tyrosine phosphatases (PTPs). PSTPIP1 is an adaptor protein that interacts with various other proteins including CD2, c-Abl, and Wiskott-Aldrich syndrome protein. We originally hypothesized that inflammation might be mediated by T cells as in classic autoimmune disease; however, we have found no changes in T cell function in patients compared to controls. Alternatively, cells of myeloid lineage may mediate PAPA syndrome. Supporting this is the report that PSTPIP1 protein interacts with pyrin, a protein expressed exclusively in myeloid cells and responsible for Familial Mediterranean Fever (FMF), an autoinflammatory disorder with clinical features similar to PAPA syndrome. We have now investigated the expression of PSTPIP1 in neutrophils and monocytes. PSTPIP1 is detected in highly purified fractions of both cell types by RT PCR and Western blotting. Interestingly a population of oversized neutrophils that appear apoptotic is detected in patients but not control samples. Using FACS analysis we have also detected a population of enlarged, HLA DRII-positive monocytes in these patients. These results are consistent with a role for myeloid cells in PAPA syndrome in which apoptotic pathways are activated, possibly mediated through pyrin. Further characterizations of these cells are in progress. Biochemical pathways identified in these experiments will reveal insights into PAPA syndrome and related inflammatory diseases.

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Identification of novel cartilage-expressed genes. *R. Pogue*¹, *E. Sebald*¹, *D. Cohn*^{1,2}, *D. Krakow*^{1,2}. 1) Pediatrics, Cedars-Sinai Medical Ctr, Los Angeles, CA; 2) Departments of Human Genetics and Pediatrics, UCLA School of Medicine, Los Angeles, CA.

To identify novel candidate genes for the osteochondrodysplasias, we performed a microarray experiment using a custom array to identify cDNA clones with particularly high levels of expression in human fetal articular chondrocytes relative to dedifferentiated chondrocytes in culture. Differential expression was then independently tested using real-time PCR. We present here eight genes with a high differential expression. Of these, two have been previously characterized (IL6R & RTP-801), two contain identifiable protein motifs (ZIP domain, SNF2 & DEXDc domains), and four are novel genes with homology to existing ESTs but no similarity to any gene of known function. One of the novel genes (designated CCL-138) has a particularly limited range of tissue expression (cartilage, hypothalamus, uterus, cochlea), making it a candidate target for experiments investigating skeletal development. Not only are these genes candidates for osteochondrodysplasia disease genes, but they also represent markers whose expression characterizes the differentiated state of chondrocytes.

Huntingtin's misfolding may hinder its export from the nucleus. *J.C. Cornett^{1,2}, H. Zhou¹, S.H. Li¹, X.J. Li¹.* 1) Dept Human Gen, Whitehead Bldg, Emory Univ, Atlanta, GA; 2) Graduate Program in Genetics and Molecular Biology.

Nuclear accumulation and aggregation of polyglutamine proteins is a common feature of polyglutamine diseases. In Huntington disease (HD), N-terminal huntingtin fragments, even without conserved nuclear localization sequences (NLS), are able to accumulate in the nucleus and form nuclear inclusions, whereas full-length mutant huntingtin is predominantly present in the cytoplasm. These facts suggest that small N-terminal huntingtin fragments may be passively translocated in the nucleus. The nuclear environment is likely to favor protein misfolding and thus retains mutant huntingtin in the nucleus. To test this hypothesis, we transfected HEK293 cells with NLS-linked huntingtin exon 1. Consequently, its concentration in the nucleus is largely determined by its export from the nucleus. NLS-huntingtin with a normal repeat (NLS-20Q) was less concentrated in the nucleus than that with an expanded repeat (NLS-150Q), suggesting that normal huntingtin is readily exported from the nucleus. Co-expression of chaperones (HSP40 and MRJ) that reduce huntingtin misfolding and aggregation significantly decreased the nuclear concentration of NLS-150Q, leading to an increase of its cytoplasmic concentration. Another chaperone (HSP70) that does not affect huntingtin aggregation showed no or much less effect on the nuclear accumulation of NLS-huntingtin. Treatments that decrease the activity of HSPs also increase huntingtin's aggregation and its nuclear accumulation. We propose that the nuclear accumulation of huntingtin is largely due to huntingtin misfolding that could affect the export of huntingtin from the nucleus.

New mutations of NaV1.1 gene encoding a neuronal sodium channel in families with GEFS+. *G. Annesi¹, A.A. Pasqua¹, S. Carrideo¹, D. Civitelli¹, P. Spadafora¹, F. Annesi¹, G. Incorpora³, A. Polizzi³, I.C. Cirò Candiano¹, P. Tarantino¹, A. Gambardella².* 1) Inst of Neurological Sciences, National Research Council, Cosenza, Italy; 2) Institute of Neurology, University of Magna Graecia, Catanzaro, Italy; 3) Institute of Pediatrics, University of Catania, Italy.

Generalized epilepsy with febrile seizures plus (GEFS+) is a familial epilepsy syndrome characterized by the presence of febrile and afebrile seizures. One of the loci for GEFS+ has been reported on chromosomal band 19q13.1, and subsequently a mutation of the voltage-gated sodium channel beta1-subunit gene has been identified in a family with GEFS+. Recently, mutations of the alpha subunit of the sodium channel, NaV1.1 on chromosome 2q24 have been reported in individuals with the GEFS+ phenotype. In this paper we studied 8 Italian families with GEFS+, by mutation analyses of genes NaV1.1 and beta1. Genomic DNA was extracted by standard methods. The 26 exons of NaV1.1 and 5 exons of SCN1B were individually amplified using primers based on intronic sequence, and PCR products were analyzed by single strand conformation polymorphism (SSCP) and sequence. In two GEFS+ families, we identified two novel mutations of the gene NaV1.1, that co-segregate with the disorder. In the remaining 6 families, screening of NaV1.1 gene revealed several single nucleotide polymorphisms (SNPs). The SNPs were found at low frequency in both GEFS+ and control populations. In the SCN1B gene we found no variants that co-segregate with GEFS+ phenotype, only one variant was identified in intron 5 (IVS5-10 C>G) which did not segregate with GEFS+ and was observed in control populations. The results of our study further support the role of Nav 1.1 gene in the etiology of this peculiar epileptic disorder. It also provides evidence of a genetic heterogeneity because several GEFS+ families did not carry any mutations in either gene. Obvious candidates include other neuronal sodium channels and proteins that interact with sodium channels.

MAP Kinase pathway in the Ts65Dn mouse model of Down syndrome. *K. Gardiner*¹, *K. O'Brien*¹, *L. Crnic*², *M. Davis*³. 1) Eleanor Roosevelt Inst, Denver, CO; 2) University of Colorado Health Sciences Center, Denver, CO; 3) The Jackson Laboratory, Bar Harbor, ME.

The Ts65Dn mouse model of Down syndrome is trisomic for ~100 genes with homologues on human chromosome 21. The phenotype of the Ts65Dn mouse includes age-related deficits in hippocampal-based learning, degeneration of basal forebrain cholinergic neurons and abnormalities of concentrations and distributions of nerve growth factor (NGF). Three observations suggest the involvement of the MAP kinase signaling pathway in the Ts65Dn and Down syndrome phenotypes: NGF signals through the MAPK pathway, hippocampal-based learning can be inhibited by inhibiting MAPK and the chromosome 21 genes - ITSN, APP, TIAM1, and SYNJ1 - influence or are predicted to influence the MAPK pathway. To test this hypothesis we have measured protein levels of the four chromosome 21 genes and several MAPK components in the brains of Ts65Dn and normal mice of ages 4-6 months. As expected the levels of chromosome 21 proteins were increased ~50% in the Ts65Dn vs normal mice. Levels of MAPK proteins, however, were variable: levels of phosphorylated ERK1/2 decreased (~25%) while levels of total ERK2 were unchanged; levels of phosphorylated Akt1 and ChAT also were decreased; levels of total ERK5 were unchanged. These data show that the MAPK pathway is perturbed in the trisomic mice and suggest a link between increased expression of chromosome 21 genes and a cellular and cognitive phenotype. These observations are being extended to mice of younger and older ages, to specific brain regions and to targets of the MAPK pathway.

Identification of Novel Genes involved in Spinal Muscular Atrophy using High Density Microarrays. *K.N. Anderson, A. Potter, C. Ponting, K.E. Davies.* Human Anatomy and Genetics, University of Oxford, Oxford, Oxon, UK.

Spinal Muscular Atrophy (SMA) is a common motor neuron disease of childhood that is usually fatal in infancy and is characterised by profound weakness and wasting. The disease is caused by mutations or deletions within the Survival Motor Neuron (SMN) gene in over 97% of cases. Although the SMN protein is known to have an essential housekeeping role in RNA metabolism, the reason for the specific motor neuron loss in the disease remains unclear.

Microarrays are a powerful tool for examining differences in gene expression profile between cDNA populations. In this study, nylon cDNA arrays spotted with 75,000 clones representing approximately 90% of the human genome were used. Primary muscle cultures were established from an SMA patient and from two age and sex matched normal controls. RNA was harvested from confluent, differentiated myotubes. ³³P dATP labelled cDNA was then hybridised onto nylon filters, the resulting signal was detected on a phosphoimager and analysed using ImageneTM and GenespringTM software. All experiments were carried out in triplicate to minimise background noise. Genes which were greater than 2 fold up or down regulated in the SMA patient sample compared to both normal controls were analysed further. Two normal control samples were used to further decrease the chance of false positive results. Cluster analysis was also carried out on the data to look for patterns of change within known gene pathways.

The SMN gene was down regulated in the SMA patient sample as expected but a number of genes involved in RNA metabolism and also synaptic transport were up regulated in the patient sample. A number of these expression differences have been confirmed using real time PCR and these results will be discussed further.

Western blot analysis is ongoing but the initial results of these microarrays suggest that this technique can be used to provide unique and exciting insights into the cellular pathology of Spinal Muscular Atrophy.

A single-base insertion in SPG3A is associated with autosomal dominant hereditary spastic paraplegia. D.

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Hereditary spastic paraplegia (HSP) comprises a clinically heterogeneous group of neurodegenerative disorders of the motor neuron system that cause progressive spasticity and weakness of the lower limbs. To date, eight autosomal dominant HSP loci have been identified, but only one gene cloned, namely SPAST on chromosome 2p22, encoding the protein spastin. Recently, mutations in a second gene, SPG3A, have been claimed to be causative in pure autosomal dominant HSP pedigrees linked to chromosome 14q11q21. We now report on a novel frameshift mutation (c.1688insA) resulting in premature translation termination of the SPG3A product atlastin, providing definitive evidence that SPG3A is truly the second disease gene in ADHSP.

Mutations of SPG4 are responsible for a loss of function of spastin, an abundant neuronal protein localized to the nucleus. *D. Charvin*¹, *N. Fonknechten*², *C. Cifuentes-Diaz*¹, *V. Joshi*¹, *J. Hazan*², *J. Melki*¹, *S. Betuing*¹. 1) Molecular Neurogenetics Laboratory, INSERM, University of Evry, E9913, Genopole, 2 rue Gaston Cremieux, CP5724, 91057, Evry, France; 2) Genoscope, Centre National de Sequenage, Evry, France.

Mutations of spastin are responsible for the most common autosomal dominant form of hereditary spastic paraplegia (HSP), a disease characterized by axonal degeneration of corticospinal tracts and posterior columns. Generation of polyclonal antibodies specific to spastin has revealed two isoforms of 75 and 80 kDa in both human and mouse tissues, with a marked tissue-specific variability of the isoform ratio. Immunoblot and immunolabelling experiments showed a nuclear localization of spastin with a high expression in neural tissues. In addition, these experiments have revealed that spastin expression is restricted to neurons and not glial cells. These data indicate that axonal degeneration linked to spastin mutations is caused by a primary defect of neurons. Protein analysis of HSP patients carrying either nonsense or frameshift spastin mutations did not reveal truncated protein. Moreover, semi-quantitative RT-PCR amplification analysis of spastin RNA revealed a reduced amount of spastin transcripts caused by instability of mutated RNA in HSP patients. Taken together, these data suggest that a dosage effect of spastin in neurons is the molecular mechanism underlying this form of HSP. Identifying agents able to induce the expression of the non-mutated spastin allele should represent an attractive therapeutic strategy in this disease.

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Roles of p53 in Huntington's Disease: possible involvement for mitochondrial function and cytotoxicity. *B. Bae, S. Igarashi, M. Fujimuro, Y. Ozeki, C.A. Ross, S.H. Snyder, A. Sawa.* Depts. of Psychiatry, Neuroscience, and Oncology Center, Johns Hopkins Univ. Sch. of Med. Baltimore, MD.

We reported that p53 was highly upregulated by mutant Huntingtin (Htt) with the expanded polyglutamine repeats both in a HD cell model (PC12 cells with Htt) and Htt transgenic animals, which conferred increased p53-mediated gene expression. We also reported that deletion of p53 increased cellular viability in HD cell model as well as normalized impaired behavior under rotarod tasks and reduced hyperactivity in rotational movements which were found in HD animal models. Here we present an additional data to support roles of p53 in HD animal models; a reduced mortality and restoration of impaired pre-pulse inhibition in mutant Htt transgenic mice when p53 was partially deleted. HD patient lymphoblasts display vulnerability in mitochondrial depolarization, which can be influenced by p53. Therefore, we have analyzed mitochondrial membrane potential as a possible downstream mechanism of Htt/p53. We observed mitochondrial depolarization in PC12 cells expressing mutant Htt, but not normal Htt, which is prior to cell death. The mitochondrial depolarization was normalized by the addition of a p53 inhibitor. Since mitochondria play a role in calcium homeostasis as well as cell death initiation, we are now studying intracellular calcium level of mutant Htt cells and response to glutamate, with or without inhibition of p53.

Autosomal Recessive forms of Axonal Charcot Marie Tooth (CMT)disease: Founder effect in LMNA and mutation spectrum in GDAP1 genes. *H. Azzedine¹, M. Tazir², N. Birouk³, G. Durosier¹, D. Ente¹, M. Salih⁴, O. Dubourg¹, A. Vandenberghe⁵, G. Stevanin¹, D. Grid⁶, A. Brice¹, E. Le Guern¹.* 1) INSERM U289, Hopital Salpetriere, 75013 Paris, France; 2) Hopital Mustapha, Sevice de Neurologie, Algiers, Algeria; 3) Hopital des specialites, Service de Neurogenetique, Rabat, Morocco; 4) Department of Paediatrics, College of Medecine, Riyadh, Saudi Arabia; 5) Hopital de l'antiquaille, Lyon, France; 6) AFM-Genethon, Paris, France.

CMT is a pathological 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 and axonal. Thirty loci and various modes of inheritance were described: autosomal dominant, X linked and autosomal recessive (ARCMT). 3 loci were reported on the axonal form of ARCMT: 1q21, 8q13 and 19q13. We selected 43 consanguineous families with axonal ARCMT including 72 patients among 193 sampled subjects. Linkage analysis and homozygosity mapping were performed using microsatellite markers spanning the 3 ARCMT regions. 15 families (35%) were linked to the 1q21 locus (LMNA gene), 12 (28%) to the 8q13 locus (GDPAD1 gene) and only one family was linked to 19q13 (2%). In conclusion, ARCMT families were rarely linked to the 19q13 locus in contrast to two other loci, which are frequent in our series. Direct sequencing of the corresponding genes was performed on linked families. In families with putative linkage to 1q21, the R298X mutation in the LMNA/C gene was predominant (73%), highly suggesting a founder effect in North Africa. In families with putative linkage to 8q13, the S194X mutation was predominant in Morocco (4/5), but rare in other regions of North Africa, also highly suggesting a founder effect. Haplotype reconstruction with markers flanking LMNA and GDAP1 are in progress to test these hypotheses. Furthermore, we identified a new mutation (R318G) in a compound heterozygote of Moroccan origin, also carrying the S194X mutation. Finally, about 32% of families were not associated with the known loci, demonstrating further genetic heterogeneity.

Holoprosencephaly : mutational screening and functional analysis of human SHH missense variants. C.

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Holoprosencephaly (HPE ; 1,2/10.000 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 aetiology of HPE. This study includes 150 unrelated nonchromosomal HPE cases (100 typical HPE, 25 atypical cases, 25 polymalformative cases). We provide clinical data regarding the subgroup of typical HPE and report 25 novel heterozygous mutations (17% for all the cases, 25% for typical HPE), 13 in Sonic hedgehog gene (SHH), 6 in ZIC2, 5 in SIX3, and 1 in TGIF. Fourteen mutations were found in familial cases whereas 11 mutations were identified in apparently sporadic cases. In addition to clear loss-of-function mutations conferred by nonsense or frameshift alteration in the coding sequence, genetic screening has revealed missense codons with less obvious functional consequences. The ability to discriminate between a loss-of-function mutation and a silent polymorphism is important for genetic testing for inherited diseases like HPE where the opportunity for prenatal diagnosis may be considered. We report here a functional test where the significance of SHH aminoacids replacements observed in the human population is tested by the C3H10T1/2 osteoblast transformation and phosphatase alkaline production under the Sonic Hedgehog action.

Spartin, the Troyer syndrome gene, suggests defective endosomal trafficking underlies some forms of hereditary spastic paraplegia. *A.H. Crosby, H. Patel, M.A. Patton, C. Proukakis, H. Cross.* Dept Medical Genetics, St George Hosp Med Sch, London, England.

Troyer syndrome (TRS) is an autosomal recessive complicated form of hereditary spastic paraplegia present at high frequency amongst the Old Order Amish (for a detailed clinical description see accompanying abstract). We have identified a single extensive Amish pedigree comprising 21 affected individuals. Having excluded known HSP loci, we used homozygosity mapping assuming a founder effect to position the TRS locus (SPG21) within a 731kb interval of chromosome 13q12.3. This region comprises eight PACs encompassing 15 complete transcripts and the first three exons of a 16th transcript. Sequence analysis of all coding exons in the interval revealed the homozygous deletion in affected individuals of a single base pair in exon four (1110delA) of a novel, highly conserved gene designated spartin. This frameshift mutation is predicted to result in the substitution of the 29 amino acid residues following the mutation, and to truncate the protein by 268 residues (fs369-398X399). Single strand conformation polymorphism (SSCP) and sequence analysis of spartin exon four reveals that this mutation cosegregates perfectly with the disease in this population. The 1110delA mutation was also confirmed in the RNA extracted from the lymphocytes of affected individuals. In order to determine if this change represents a polymorphism, SSCP was used to screen 760 normal control chromosomes of mixed Caucasian (80%), Asian (10%) and African (10%) descent, as well as 44 chromosomes originating from the same Amish community but not closely related to the TRS cases. No 1110delA mutations were detected. By comparative sequence analysis, we have identified three novel functional domains in spartin. Importantly one of these (MIT) is also present in spastin, mutated in more common forms of HSP, as well as in molecules that may function in endosomal trafficking. This finding reveals a functional link between two molecules mutated to result in HSP and highlights a putative mechanism responsible for some forms of neurodegeneration.

Genetic heterogeneity in Silver syndrome. *C.D. Fenske¹, T.T. Warner², H. Patel¹, M.A. Patton¹, C. Proukakis¹, A.H. Crosby¹.* 1) Medical Genetics Unit, St George's Hosp Medical, London, England; 2) Department of Clinical Neurosciences, Royal Free and University College Medical School, London, UK.

The hereditary spastic paraplegias (HSPs) are a group of clinically and genetically heterogeneous neurodegenerative disorders of the motor system. Silver syndrome (SS) is an autosomal dominant form of HSP in which the spasticity is associated with marked amyotrophy and weakness of the small muscles of the hands. We previously reported the localisation of the SS locus (SPG17) to a 13cM interval on chromosome 11q12-q14 and exclusion of two candidate genes (CNTF and CCS) as being causative of the disease. Here we report the identification of a second pedigree mapping to the SPG17 locus, and the exclusion of three additional families. This demonstrates further locus heterogeneity in HSP, even in this rare complicated form of the disease.

Single Cell Genomic DNA Analysis via Laser Capture Microscopy: Evaluation of LOH in the Development of Cerebral Cavernous Malformations. *K.A. Gee¹, L. Nowak¹, J. Zabramski², S. Coons³, E.W. Johnson¹.* 1) Neurogenetics/Neuropharm; 2) Neurosurgery; 3) Neuropathology, Barrow Neurological Inst, Phoenix, AZ.

The principle gene thought to be responsible for familial cerebral cavernous malformations (CCMs), CCM1, was identified as KRIT1. KRIT1 is a member of the Krev-1/rap1A/Ras family of proteins that play a role in tumor suppression/cell division suppression. CCM1/KRIT1 is believed to be causative in approximately 50-70% of familial CCM cases. To date no one has identified any individual homozygous for the mutated KRIT1 gene, allowing the possibility that homozygosity might be a lethal condition. The loss of KRIT1 function in vascular cells due to a mutation would in effect remove this suppression allowing for a tumor-like proliferation of endothelial cells. A reasonable hypothesis that could explain the focal nature of CCMs suggests that these "vascular tumors" may develop due to loss of the wild-type KRIT1 allele in the developing cerebral vasculature. Laser Capture Microscopy (LCM) will be used to address the idea that this loss of heterozygosity (LOH) in KRIT1 in cerebrovascular endothelial cells is a trigger for the development of CCMs. LCM allows the harvesting of definitively tagged cells with a resolution as small as individual nuclei. We will selectively harvest endothelial cells from CCM tissue removed during surgery and compare the KRIT1 alleles found there with the patients genomic haplotype derived from circulating lymphocytes and/or from non CCM derived endothelial cells where available. LCM brings to gene expression studies what quantitative autoradiography brought to pharmacological studies in the 1980's: substantially increased anatomical resolution. Regionally discrete changes are often missed when large areas of tissue are examined since the robust "normal" signal present in most of the tissue masks any neuroanatomically discrete variations that might be of interest. Whatever the mechanism, these data point to a key role for the KRIT1 and the Krev-1/rap1a pathway in the complex process of angiogenesis, and potentially, in other forms of cerebrovascular disease.

Chudley-Lowry syndrome results from absence of the XNP protein. *F.E. Abidi¹, A.E. Chudley², S. Daniels¹, T. Moss¹, H.A. Lubs³, R.E. Stevenson¹, C.E. Schwartz¹.* 1) J.C. Self Research Institute, Greenwood Genetics Center, Greenwood, SC; 2) Genetics Health Sciences Center, FE229-820, Winnipeg, MB R3A IR9, Canada; 3) University of Miami School of Medicine, Genetics Division/Department of Pediatrics, PO Box 016820/D-820, Miami, FL.

Chudley-Lowry syndrome (MIM # 309490) is an X-linked recessive condition characterized by moderate to severe mental retardation, short stature, mild obesity, hypogonadism, a depressed nasal bridge and anteverted nares (Chudley et al., 1988). The family consists of 3 affected males in two generations. Marker analysis had localized the gene between Xp21-Xq26. An obligate carrier was tested for X-inactivation (XI) and showed a highly skewed (95:5) pattern. The combination of the clinical phenotype, consistent with XLMR-hypotonic facies, the skewed XI and the localization covering Xq13, suggested the existence of an XNP mutation might be the cause of the syndrome. Eighty percent of all the mutations reported in the XNP gene are found in the zinc finger and the helicase domains of this gene. Unfortunately analysis of these domains in the proband of Chudley-Lowry family did not detect any mutation. Therefore, we designed a RT-PCR based strategy to expand the study to the rest of the gene. The RT-PCR analysis revealed a nonsense mutation (C109T) in exon 2 of the XNP gene, giving rise to a stop codon at position 37 (R37X). As a result, the affected males completely lack the XNP protein, although the presence of a premature stop codon does not affect the stability of the altered XNP transcript. Our findings suggest that Chudley-Lowry syndrome is yet another syndrome that is allelic to the XLMR-hypotonic facies syndrome. Interestingly, the lack of the XNP protein has not resulted in as a severe phenotype in the family as missense mutations. This would indicate that the absence of XNP protein appears cause less disruption of development and brain function than some altered forms of the XNP protein. One explanation is that some XNP mutations may be exerting a dominant-negative effect.

Familial epilepsy incorporating "benign" infantile convulsions: phenotypic overview, linkage to chromosome 19q and analysis of candidate genes. *S.A. McKee, A.E. Hughes.* Dept of Medical Genetics, Queen's University Belfast, Northern Ireland.

PURPOSE: Epilepsy affects 2-4% of people at some point in their lives. Genetic factors are important in idiopathic epilepsy, but kindreds displaying mendelian inheritance are rare. Several genes (notably ion channels) have been implicated in some such families, offering insights into more common forms of epilepsy. We studied a large Irish family with a history of seizures, consistent with autosomal dominant inheritance.

METHODS: 32 consenting family members were interviewed and examined, DNA was obtained, and medical records were consulted. Genome-wide linkage analysis was undertaken, adopting a stringent affected-only data model. Candidate genes were sequenced.

RESULTS: The most consistent phenotype was of generalised seizures beginning ~3mo, lessening by ~2yr. Many subjects had seizures persisting into childhood or adulthood. The severity of seizures appeared to be greater in males in the most recent generation, suggesting anticipation and sex-bias. 18 subjects were classed as affected, and there were 4 obligate gene carriers. A LOD score of 3.62 was found at D19S220 on chromosome 19q. No other regions of significant linkage were detected in the genome. Nearby candidate genes included the ion channels SCN1B and KCNK6; no mutations were found in these genes. A polyglutamine tract in the neuronally-expressed NUMBL gene did not show evidence of expansion.

CONCLUSIONS: The phenotype in this family is different from recognised genetic epilepsy syndromes. It bears similarities to benign familial infantile convulsions (BFIC), but there are important differences, particularly in the persistence of seizures into later life, possible anticipation, and the high degree of variability. One form of BFIC has previously been mapped more proximally on 19q; further work is required to determine whether these seizure disorders are allelic, or due to different genes.

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Presentation of one family Affected with HIBM,a report from Iran. *S. Joughehdoust, Y. Shafeghati, M. Ataii, M. Houshmand.* NRCGEB, Tehran, tehran, Iran.

Hereditary Inclusion Body Myositis is a chronic progressive muscle disorder .Inheritance is autosomal recessive. It is started with insidious onset in second or third decades of life and associated with distal muscle weakness in the upper extremities and proximal muscle weakness in the lower extremities. Our patient is 24 years old girl that suffers from muscular weakness. Positive findings in her physical exam are mild weakness of neck flexors,deltoid and scapular muscles and in lower extremity , severe iliopsoas , gluteal ,plantar and dorsiflexor weakness. The quadriceps however is normal.EMG shows myopathic feature and muscle biopsy is compatible with muscular atrophy,neurogenic .All of the above findings are typical for HIBM. HIBM is prevalent in Iranian Jews.The patient is from an unconsanguineous marriage and her grand parents were from Iranian Jewish ancestors. In our lab we are analyzing a mutation in the 3UTR region of CNTFR mRNA gene,this gene is a 305 bp long .The method is PCR/RFLP. To detect homozygote or carrier state all members of her family will be examined for this mutation. In spite of its autosomal recessive pattern of inheritance we are planning to find a relation between mitochondrial myopathies mutations and HIBM, because the basis of both disorders is lack of ATP synthesis.

Molecular characterization of *ZFHX1B* in patients with SIP1 deficiency. *N. Ishihara*^{1,7}, *K. Yamada*¹, *Y. Yamada*¹, *K. Miura*², *K. Ohya*⁴, *A. Nakayama*⁵, *M. Yoneda*⁶, *M. Nagaya*³, *N. Wakamatsu*¹. 1) Dept Genet, Inst Dev Res, Aichi Human Service Ctr, Kasugai, Aichi, Japan; 2) Dept Pediatr Neurol, Centl Hosp, Aichi Human Service Ctr, Kasugai, Aichi, Japan; 3) Dept Pediatr Surg, Centl Hosp, Aichi Human Service Ctr, Kasugai, Aichi, Japan; 4) Dept Pediatr, Aomori Centl Hosp, Aomori, Japan; 5) Dept Embryol, Inst Dev Res, Aichi Human Service Ctr, Kasugai, Aichi, Japan; 6) 2nd Dept Inter Med, Fukui Med Univ, Fukui, Japan; 7) Dept Pediatr, Nagoya Univ Sch Med, Nagoya, Japan.

The Hirschsprung disease-mental retardation syndrome (MIM 235730) is genetically and clinically quite heterogeneous. Recently, we identified mutations in *ZFHX1B*, encoding Smad-interacting protein 1 (SIP1), associated with the autosomal dominant form of this disease. Patients who have nonsense or frameshift mutations of *ZFHX1B* in one of alleles typically show profound mental retardation, delayed motor development, microcephaly and facial dysmorphism, with various combinations of Hirschsprung disease, epilepsy, congenital heart diseases and abnormal brain findings. We have been continuing molecular analysis of *ZFHX1B* and identified an insertion of 382-bp of AluYa5 in exon 8 (390fs430X) as well as a novel insertion of 19 nucleotides, instead of the 14-bp deletion (465fs467X) found in typical cases. Moreover, a 3-bp deletion mutation (N99del) was also identified in one allele from a patient suffering moderate mental retardation and megacolon. To investigate whether the mild clinical features observed in the patient with the 3-bp deletion in *ZFHX1B* might have been caused by dysfunction of transcriptional repression of SIP1, we constructed expression vectors containing full length *ZFHX1B* cDNAs with and without mutations identified in patients with SIP1 deficiency. The aim is to demonstrate molecular mechanisms of mental retardation caused by SIP1 deficiency by co-transfection of *ZFHX1B* cDNAs and chimeric constructs of the E-cadherin promoter region driving luciferase into HEK293 cells.

g-synuclein gene alternation in a case of dementia with Lewy bodies (DLB). *H. Ohtake*¹, *O. Onodera*^{1,2}, *S. Igarashi*^{1,2}, *M. Saito*¹, *H. Takahashi*³, *A. Ishikawa*⁴, *J. Idezuka*⁵, *M. Wakabayashi*⁵, *B. Giasson*⁶, *V.M.-Y. Lee*⁶, *J.Q. Trojanowski*⁶, *S. Tsuji*¹. 1) Dept of Neurol; 2) Molecul Neurosci; 3) Neuropathology, Brain Res Inst, Univ Niigata, Niigata, Japan; 4) Niigata Nat Hosp, Niigata, Japan; 5) Ojiya Sakura Hosp, Niigata, Japan; 6) Center for Neurodegenerative Dis Res and Dept of Patho and Lab Med, Univ Pennsylvania, Philadelphia, PA.

DLB and Parkinson's disease (PD) are characterized by intracellular inclusions, Lewy bodies. α -Synuclein (SNCA) has been shown to be a major component of Lewy bodies and to have a property of forming fibrils. Despite the amino-acid sequence similarities among the synuclein family genes, b- and g-synucleins (SNCB, SNCG) tend to be more soluble compared to SNCA. It has been suggested that SNCB and SNCG inhibit fibrillation of SNCA. Two missense mutations of SNCA have been reported in AD-PD. In 2001 Limprasert *et al.* reported a DLB case with a missense mutation in SNCB. We hypothesized that loss of the physiological function of SNCB or SNCG to protect fibril formation of SNCA can also cause DLB or PD. We analyzed the coding sequences of synuclein family genes in 3 pedigrees (2 with AD inheritance and one with consanguineous marriage) and 2 sporadic cases. Among them, we identified an A257G missense mutation of SNCG in an 81-year-old woman without family history, but no mutations in others. This mutation results in substitution of glycine for glutamic acid and expected to be more insoluble. Interestingly, the amino acid of SNCA corresponding to the mutated codon in SNCG is glycine, identical to the glycine in the mutant SNCG at the same codon, and furthermore, this substitution is located at the position corresponding to the region near the 11 hydrophobic residues of SNCA, which plays important roles in fibrillation of SNCA. These data raise the possibility that the mutant SNCG promote fibrillation of not only SNCA but also SNCG. Since detailed information on the family members are unavailable, and her offsprings were too young to be evaluated for disease presentations, further follow-up studies will be required. We propose that this mutation in SNCG is potentially responsible for pathogenesis of DLB.

A simple method to confirm clinical diagnosis of autosomal recessive Spinal Muscular Atrophy by Denaturing High Performance Liquid Chromatography (DHPLC). *R. Mazzei¹, F.L. Conforti¹, M. Muglia¹, T. Sprovieri¹, A. Magariello¹, A. Patitucci¹, A.L. Gabriele¹, A. Quattrone^{1, 2}.* 1) Institutes of Neurological Sciences, National Research Council, Mangone , CS, Italy; 2) Institute of Neurology, School of Medicine of Catanzaro, Italy.

Spinal Muscular Atrophy (SMA) is characterized by degeneration of motor neurons in the spinal cord, causing progressive weakness of the limbs and trunk, followed by muscle atrophy. SMA is one of the most frequent autosomal recessive diseases, with a carrier frequency of one in 50 and the most common genetic cause of childhood mortality. Recessive SMAs are caused by mutations in the Survival Motor Neuron gene (SMN). There are two nearly identical copies of this gene, the telomeric SMN (SMNt) and its centromeric counterpart (SMNc), present on chromosome 5q13; however, only the SMNt gene is affected in SMA. Furthermore, small deletions or point mutations have been found in patients in whom SMNt is present. In this study, we describe a new method to detect SMA gene deletion by Denaturing High Performance Liquid Chromatography (DHPLC), which is also simple to perform, but is faster and more specific. As telomeric and centromeric copies of exon 7 of the SMN gene differ only by a single base pair, denaturation and slow renaturation of PCR products of this exon leads to formation of heteroduplexes and homoduplexes. The same also occurs in exon 8. Therefore, by using DHPLC we should obtain a different resolution in normal controls from that in patients carrying the deletion. The main advantages of our approach are that it is simple to perform, and is faster and less expensive than previously described methods. Furthermore, it leads to clear and unambiguous results.

Deficiency of α -dystroglycan in muscle-eye-brain disease. *H. Kano*¹, *K. Kobayashi*¹, *R. Herrmann*², *T. Voit*², *H. Topaloglu*³, *T. Endo*⁴, *T. Toda*¹. 1) Div Functional Genomics, Dept Post-Genomics and Diseases, Osaka Univ Grad Sch Med, Osaka, Japan; 2) Dept Pediatrics and Pediatric Neurology, Univ Essen, Essen, Germany; 3) Dept Pediatric Neurology, Hacettepe Children's Hospital, Ankara, Turkey; 4) Glycobiology Research Group, Tokyo Metropolitan Institute of Gerontology, Foundation for Research on Aging and Promotion of Human Welfare, Tokyo, Japan.

α -Dystroglycan is a component of the dystrophin-glycoprotein-complex (DGC), which is the major mechanism of attachment between the cytoskeleton and the extracellular matrix. Muscle-eye-brain disease (MEB) is an autosomal recessive disorder characterized by congenital muscular dystrophy, ocular abnormalities and lissencephaly. We recently found that MEB is caused by mutations in the protein *O*-linked mannosyl transferase (POMGnT1) gene. POMGnT1 is a glycosylation enzyme that participates in the synthesis of *O*-mannosyl glycan, a modification that is rare in mammals but is known to be a laminin-binding ligand of α -dystroglycan. We examined skeletal muscle tissue samples from MEB patients to evaluate the state of the DGC and to search for proteins that are modified by POMGnT1. Immunohistochemistry and immunoblotting were performed using anti-laminin α 2 chain, anti- α -dystroglycan, anti- β -dystroglycan, and anti-dystrophin antibodies. Immunoreaction to the anti- α -dystroglycan antibody was barely detectable on the surface membranes of skeletal muscle fibers in MEB patients relative to the control. On the other hand, immunoreactivity of laminin α 2 chain, β -dystroglycan and dystrophin proteins were similar between MEB patients and the control. Immunoblot analysis of protein extracts from muscle tissue gave consistent results in which the muscle specimen from the MEB patient showed a lack of α -dystroglycan expression, although normal levels of β -dystroglycan were observed. This finding suggests that α -dystroglycan is a potential target of POMGnT1 and that altered glycosylation of α -dystroglycan may play a critical role in the pathomechanism of MEB and some other forms of muscular dystrophy.

Mutations in the TGIF gene and SIX3 gene in patients with holoprosencephaly. *J. Herbergs¹, H.W. Moerland¹, J.J. Van der Smagt³, G.M.S. Mancini², M. Van Haelst², H.J.M. Smeets¹.* 1) Department of Clinical Genetics, Academic Hospital Maastricht, Maastricht, The Netherlands; 2) Department of Clinical Genetics, Erasmus University, Rotterdam, The Netherlands; 3) Leiden University Medical Hospital, Leiden, The Netherlands.

Holoprosencephaly (HPE) is a common, severe malformation of the brain that involves abnormal formation and septation of the developing central nervous system. In mild HPE microcephaly, a single central incisor, hypotelorism or other craniofacial findings can be present with or without associated malformations. Mutations in several genes are known to cause HPE. In our lab four genes known to be involved in the pathogenesis of HPE are initially analysed for pathogenic mutations, namely SHH, ZIC2, SIX3 and TGIF. In a small series of patients a new truncating mutation was detected in the SIX3 gene and a missense mutation in the TGIF gene. The patient with a SIX3 mutation has semilobar HPE with cleft palate, while the patient with a TGIF mutation presented with more severe HPE. The mutation in the TGIF gene could affect a region of the protein constituting a repression domain, which is dependent on HDAC and contains a SMAD-interacting region. The mutation, however, was also detected in one of the parents, presenting hypotelorism and microcephaly.

Gene expression profiling of SMA mouse models using cDNA microarray. *R. OLASO¹, V. JOSHI¹, N. PAULINE¹, N. ROBLOT¹, G.M. LATHROP², J. MELKI¹.* 1) Molecular Neurogenetics Laboratory, INSERM E.9913, Université d'Evry, GENOPOLE, 91057, Evry, France; 2) Centre National de Genotypage, Evry, France.

Spinal Muscular Atrophy (SMA) is a common autosomal recessive neuromuscular disorder characterized by degeneration of motor neurons leading to progressive paralysis with muscular atrophy. Mutations of the Survival of Motor Neuron gene (SMN1) are responsible for SMA. Previous studies have suggested that SMN protein is implicated in several processes. However, the molecular pathway linking SMN defect to SMA phenotype remains to be elucidated. The Cre-loxP recombination system has been used to direct deletion of the murine *Smn* exon 7 to either neuronal or skeletal muscle cells. Both neuronal and muscular mutant mice display severe muscle paralysis leading to early death through motor neuron or skeletal muscle degeneration respectively. These data indicate that both neurons and skeletal muscle are involved in SMA pathogenesis. To refine the degenerative process in SMA and identify the targets of SMN1 gene defect, we have undertaken a comparative gene expression study using cDNA microarray. We compared targeted tissues from control and mutant mice (either neuronal or muscular) at different stages of the disease. Skeletal muscle or spinal cord mRNA extracted from control and SMN mutant mice were reverse-transcribed, labelled with Cy5 or Cy3 dyes and hybridized to the array. For each age (10, 20 and 30 day-old), 3 couples of mutant/control animals were used. We have selected genes over or under-expressed at least 1.9 fold in 3 experiments. Preliminary results showed that less than 200 out of 8000 genes examined were differentially expressed in skeletal muscle of 20 day old muscular mutant mice. We are currently ordering all these genes by functional families. As expected, several muscle specific genes were underexpressed in the mutant mice. Comparing gene expression profiling in neuronal and muscular SMN mutant mice at different ages should allow to reduce the number of candidate genes. This study should contribute to clarify the physiopathology of SMA, the function of SMN and should help to design potential targeted therapeutic molecules.

P172H MUTATION IN THE TM4SF2 GENE ACCOUNTS FOR 1% OF NON-FRAGILE X MENTAL RETARDATION IN BRAZILIAN PATIENTS. *C.M.C. Maranduba^{1,2}, A.M. Vianna-Morgante¹, M.R. Passos-Bueno¹.* 1) Centro de Estudos do Genoma Humano, Departamento de Biologia, Universidade de São Paulo, São Paulo, Brasil; 2) Centro em Interunidades em Biotecnologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brasil.

X-linked mental retardation (XLMR) affects approximately 1 in 600 males, comprising (1) syndromic forms (MRXS), among which the fragile X syndrome is the most prevalent of the 68 syndromes so far described, and (2) nonsyndromic forms (MRX). To date, eight genes have been found to be associated with MRX: FMR2, OPHN 1, GDI1, PAK3, TM4SF2, ARHGEF6, VCX-A and IL1RAPL. IL1RAPL and TM4SF2 mutations appear to account for 5-10% of MRX, while the other mutations are associated with less than 1% of the cases. We are presently screening the coding region and the exon-intron boundaries of IL1RAPL and TM4SF2 genes, through SSCP analysis and sequencing, in 105 mentally retarded male patients (25 familial and 80 isolated cases) who were shown not to carry the fragile X mutation. Four of the eight known exons of the IL1RAPL gene were analyzed in 50 patients, and two altered migration patterns were detected after SSCP, sequencing being under way. All the seven exons of the TM4SF2 gene were analyzed in 96 patients. A CA transversion at the nucleotide c.515 (P172H) was detected, which was confirmed by the SNUPE test (Amersham Biotech). This change was maternally inherited and was not carried by the healthy brother of the patient. Our survey of 100 control chromosomes did not reveal this mutation, that was first described in a familial MRX case by Zemni et al. (Nat. Genet. 24, 167 2000). Our results adds to the indication that the P172H mutation is pathogenic, with a prevalence of about 1% among non-fragile X mental retardation cases in our sample. Therefore, testing for the P172H mutation in the TM4SF2 gene should be considered in the routine diagnosis of mentally retarded individuals. Supported by FAPESP, PRONEX and CNPq.

A worldwide distribution and a broader clinical spectrum of muscle-eye-brain disease: a genotype-phenotype

correlation. *K. Kobayashi¹, K. Taniguchi¹, K. Saito², H. Yamauchi³, D.K. Jin⁴, E. Parano⁵, R.V. Coster⁶, H.*

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Muscle-eye-brain disease (MEB) is an autosomal recessive disorder prevalent in Finland, which is characterized by congenital muscular dystrophy, brain malformation (type II lissencephaly), and ocular abnormalities. Since the phenotype has a substantial overlap with those of Fukuyama-type congenital muscular dystrophy (FCMD) and Walker-Warburg syndrome (WWS), these three diseases are considered to be caused by a similar pathomechanism. Recently, we found that MEB is caused by mutations in the protein *O*-linked mannose b1,2-*N*-acetylglucosaminyltransferase 1 (POMGnT1) gene. We describe here the identification of seven novel mutations in six of not only non-Finnish Caucasian but also Japanese and Korean patients with suspected severe FCMD, MEB, or WWS. Including the seven mutations reported previously, all 13 mutations are dispersed along the whole POMGnT1 gene. A weak correlation between the location of mutation and clinical severity was observed; patients with mutations in the vicinity of the 5 terminus of the POMGnT1 coding region show relatively severe symptoms such as hydrocephalus, while patients with mutations in the vicinity of the 3 terminus have milder phenotypes. Our results suggest that MEB patients may exist in many population groups outside of Finland with a worldwide distribution beyond our expectations, and that the clinical spectrum of MEB is broader than recognized previously. These emphasize the importance of considering MEB and searching the POMGnT1 mutations, in WWS and in the worldwide outside of Finland.

Mutational Analysis and Identification of Differential Methylation Patterns in the SGCE Gene in Patients with Myoclonus Dystonia. L. Liu¹, P. de Carvalho Aguiar¹, R. Saunders-Pullman², C. Klein³, S. Bressman², L. Ozelius¹ and The Myoclonus-Dystonia Study Group. 1) Molecular Genetics, Albert Einstein College of Med, Bronx, NY; 2) Department of Neurology, Beth Israel Medical Center, New York; 3) Departments of Neurology and Human Genetics, Medical University of Lbeck, Lbeck, Germany.

Myoclonus-dystonia (M-D) is an autosomal dominant disorder characterized by myoclonic jerks and dystonia that are often responsive to alcohol. In the majority of families, the disease has been linked to chromosome 7q21 while loss of function mutations in the gene for epsilon-sarcoglycan (SGCE) have been reported. Mutation analysis was performed on the SGCE gene in families with M-D using denaturing high performance liquid chromatography (DHPLC). In total, we identified 20 mutations including: 6 frameshift mutations predicted to generate truncated proteins; 3 nonsense mutations resulting in the insertion of immediate stop codons; 5 splicing mutations expected to delete specific exon from the protein and 6 missense mutations. All six missense mutations were not seen in 500 control chromosomes and each is predicted to change a conserved amino acid in the protein. The SGCE gene is imprinted in mice. Pedigree analysis in M-D families showed a significant difference in penetrance according to the parental origin of the disease allele, suggesting that SGCE may be imprinted in humans as well. Differences in methylation were observed in the 5' UTR CpG rich region of the SGCE gene by Southern blot analysis using methylation sensitive enzymes demonstrating differential expression of the two parental alleles. In addition to lymphoblastoid lines, brain tissue was also used on the Southern blots and revealed the same imprinting pattern. This indicates that methylation of the promoter region of the SGCE gene may result in imprinting and that this mechanism operates in at least two different tissue types.

Deletion breakpoint analysis in a patient with Pelizaeus-Merzbacher disease (PMD) and comparison with duplications. *G.M. Hobson¹, C.M. Ritterson¹, T.D. Bird², W.H. Raskind², J.Y. Garbern³, K. Sperle¹.* 1) A I duPont Hosp Children, Wilmington, DE; 2) University of Washington, Seattle, WA; 3) Wayne State University, Detroit, MI.

Pelizaeus-Merzbacher disease (PMD) is an X-linked recessive dysmyelinating disorder of the central nervous system. Approximately 60% of cases are due to duplication of a genomic region that includes the PLP1 gene. While PLP1 gene duplications vary in size and breakpoint, most are tandem and intrachromosomal in origin. Another 15% to 25% are due to point mutations. Only two complete deletions of the PLP1 gene have been reported, one of >73 kbp determined by FISH analysis using BACs, and the other of >29 kbp determined by Southern blot analysis. A probable grandpaternal origin has been reported for duplications and for the deletion of >73 kbp. The disease phenotype of the patient with a deletion of >73 kbp was described as similar to that of duplication patients. The phenotype of patients with the deletion of >29 kbp is less severe than that of duplication patients, and is characterized by a longer time interval before requiring a wheelchair. In addition to the central nervous system findings, patients with deletions have peripheral neuropathy. Using sequence information available from the Human Genome Project, we have designed primer pairs in the genomic region around the PLP1 gene and performed breakpoint analysis of the deletion of >29 kbp by quantitative comparative multiplex PCR. We determined that the deleted region is >180 kbp and it includes at least one gene in addition to PLP1, RAB9-like, a member of the Ras superfamily. The deletion data will be refined and compared with duplication data of patients who have duplication endpoints nearby. Deletion and duplication breakpoint analyses and eventual determination of the genomic sequence at the breakpoints will provide insight into the molecular mechanisms involved.

Glycine receptor mRNA level correlates with phenotype severity and downstream gene changes in spastic and spasmodic murine models: an expression profiling study. *A. Molon, S. Di Giovanni, F. Murillo, E.P. Hoffman.*
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Glycine is the major inhibitory neurotransmitter in the spinal cord and brainstem. The glycine receptor (GlyR) is composed by two types of subunits: a and b. In humans, mutations in the GlyR subunit genes cause hereditary hyperekplexia, an autosomal dominant disorder. Two murine homozygote mutants are models for GlyR mutations: the spasmodic (Spd) mouse carries a mutation in the a subunit (Ala52Ser), while the spastic (Spa) mouse harbors an insertion (LINE 1) in the b subunit. Affected littermates show a variable phenotype, ranging from limb paralysis (severe), to mild startle response (mild). In order to define the relationship between mutation type, glycine mRNA levels, downstream pathogenic events, and the variable clinical phenotype, we expression profiled spinal cords of both mutants, showing different levels of phenotype severity, using Affymetrix MU74A v2 array including 12478 probe sets. Specific RNAs were verified by QMF-RT-PCR using infrared primers. The severe spastic animals showed widespread expression changes in the spinal cord, while the mild animals were similar to normal profiles. We found that GlyR mRNA levels correlated with severity of phenotype. In particular, GABA and glutamate receptors and genes involved in their transport were significantly decreased together with profiles associated with neuritogenesis and lipid biosynthesis; these functional groups were shared with experimentally-induced mild spinal cord damage. Cell cycle progression genes (G2/M phase) in inherited spinal cord damage (Spa) were differentially induced relative to spinal cord trauma (G1/S stage). The induction of cell cycle checkpoints is likely detrimental to neuronal viability. Our results suggest that variations in the level of GlyR transcription may determine the severity of phenotype. Further, we identify gene transcriptional pathways shared between a genetic defect and environmental injury, while other pathways are specific to one type of injury and are associated with a downstream transcriptional cascade that may reflect and mediate a generalized metabolic neuronal depression.

Tau pathology and motor disturbances in the human tau P301S transgenic mouse model. *J.R. Murrell, M. Takao, B. Ghetti.* Dept of Pathology, Indiana Univ Sch Med, Indianapolis, IN.

Neurofibrillary pathology with abnormal microtubule-associated protein tau (gene = MAPT; protein = tau) is one of the main pathological lesions of several types of dementia including Alzheimer disease, Pick disease and progressive supranuclear palsy. Studies of families with frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) have shown that mutations in MAPT cause diverse but mutation-specific lesions in the adult brain. Mutations in MAPT impair tau function, promote tau fibril formation, disrupt normal MAPT gene splicing and affect the normal ratio of 3:4 repeat-containing tau. Though some information is known about the effects of tau mutations, the actual mechanisms leading to neurodegeneration still need to be elucidated so that therapeutic measures can be developed. In order to study these tau-induced mechanisms of neurodegeneration, a transgenic mouse model with the human P301S tau mutation was created. This mutation was chosen because of the early onset (late 20s), unique pathology and variable clinical phenotype seen in humans. The human tau transgene is under the control of the mouse prion promoter. Two founder lines show above normal expression of the human tau gene. Hemizygous and homozygous animals from these founders show both motor and behavioral deficits at ages 11 months and 6 months, respectively. Tau positive inclusions were observed in the cingulate, somatosensory, motor, and entorhinal cortices as well as the hippocampus, caudate nucleus, putamen, cerebellum, midbrain, pons, medulla, and the anterior and posterior gray horns of the spinal cord. Thioflavin S staining suggests the presence of insoluble tau deposits. This animal represents a good model of tauopathy and should prove to be a valuable tool for studying the stages of neurodegeneration and testing potential therapeutic interventions.

Transgenic mouse model of spinal and bulbar muscular atrophy. *M. KATSUNO, H. ADACHI, A. KUME, G. SOBUE.* NEUROLOGY, NAGOYA UNIVERSITY GRADUATE SCHOOL OF MEDICINE, NAGOYA, AICHI, JAPAN.

Spinal and bulbar muscular atrophy (SBMA) is an X-linked late-onset motor neuron disease characterized by proximal muscle atrophy, weakness, contraction fasciculation, and bulbar involvement. Heterozygous female carriers are usually asymptomatic, although some express subclinical phenotypes. The molecular basis of SBMA is the expansion of a trinucleotide CAG repeat in the first exon of the androgen receptor (AR) gene, which encodes the polyglutamine (polyQ) tract. The presence of nuclear inclusions (NIs) is a pathologic hallmark of SBMA as well as most other polyQ diseases. Previously reported transgenic (Tg) mouse models of SBMA showed progressive motor deficits and NIs, although no sexual difference of phenotypes was demonstrated. In the present study, we generated Tg mice carrying a full-length AR containing 97 CAGs under the control of a cytomegalovirus enhancer and a chicken - actin promoter. Three of 5 lines exhibited small body size, short life-span, progressive muscle atrophy and weakness as well as reduced cage activity; all of which were markedly pronounced and accelerated in the male Tg mice, but not observed or far less severe in the female Tg mice regardless of the line. Western blot analysis revealed nuclear accumulation of mutant AR, and NIs were demonstrated in the neurons of the spinal cord, cerebrum, cerebellum, brain stem, and dorsal root ganglia as well as non-neuronal tissue such as the heart and muscle; these findings were also significant in male Tg mice, whereas female showed subtle abnormalities. Our findings reiterate the importance of nuclear localization of the mutant protein in the disease mechanism, and provide profound insight to the pathophysiology of SBMA.

An *in silico*-based approach for identification of a novel gene for Charcot-Marie-Tooth disorder. *G.M. Saiji*¹, *H. Takashima*¹, *J.R. Lupski*^{1,2}. 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Pediatrics, Baylor College of Medicine, Houston, TX.

Thirteen genes and sixteen genetic loci have been associated with Charcot-Marie-Tooth (CMT) and related disorders. In order to identify candidate genes in these sixteen regions, we designed an *in silico*-based approach. Using the tools of bioinformatics, we identified and mapped putative paralogs of the thirteen known CMT genes. Our hypothesis was that paralogs, which fall, in these sixteen critical regions should have a good potential as candidate genes for CMT. Using this *in silico* strategy, we identified six such positional candidates. One of these, *EMP3*, has already been ruled out (*Am J Hum Genet* **68**, 269-274). To validate these results, the most promising candidate, based on similarity score, was analyzed by DHPLC and sequencing for DNA sequence variation in our panels of CMT patients. So far, two mutations have been identified in this gene. This has thus validated our *in silico*-based approach for the identification of novel CMT genes. This strategy could thus likely be extended for the identification of disease-causing genes associated with other genetic disorders.

A mouse model of cerebral cavernous malformations generated by targeted mutation of the mouse *Ccm1* (Krit1) gene. *N.W. Plummer*¹, *M.W. Becher*², *D.A. Marchuk*¹. 1) Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC; 2) Department of Pathology, University of New Mexico Health Sciences Center, Albuquerque, NM.

Cerebral cavernous malformations (CCM) are lesions in the central nervous system consisting of aggregates of enlarged capillary-like vascular channels without intervening brain parenchyma. Hemorrhage of CCMs can result in migraines, seizures, or lethal stroke. Three inherited forms of CCM have been mapped, and the mutated gene has been identified for *CCM1*. In order to develop an animal model of CCM, we have mutated the mouse *Ccm1* (Krit1) gene by homologous recombination in embryonic stem cells. The fifth and sixth coding exons were replaced by an IRES-LacZ cassette to facilitate analysis of *Ccm1* expression patterns. RT-PCR analysis of heterozygous and homozygous embryos confirmed that no wild-type transcripts are produced by the mutant allele. Heterozygous mutant mice are viable and fertile, but homozygotes die by embryonic day 11.0. Brain sections from heterozygous animals were stained with X-Gal to test the hypothesis that *Ccm1* is expressed in the vasculature of the brain. Surprisingly, the most intense staining was in the epithelial cells of the ependyma, with weaker staining in the choroid plexus. The ependymal staining is interesting, in light of recent observations that tight junctions of the blood-brain barrier are absent in CCMs. Ependymal tight junctions contribute to the barrier between brain parenchyma and cerebrospinal fluid. Subtle morphological abnormalities were observed in the choroid plexus of heterozygous mice, and scattered, abnormally dilated blood vessels were present in year-old heterozygotes, suggesting that these mutants will be a useful animal model of CCM.

A Novel Mouse Model of Infantile Neuroaxonal Dystrophy. *S.M. Parker¹, D.M. Bouley², J. Jones McIntire², E. Hadar², R.J. Coryell¹, K.H.L. Ching¹, S.J. Hayflick¹.* 1) Departments of Molecular and Medical Genetics, Pediatrics and Neurology, Oregon Health & Science University, Portland; 2) Department of Comparative Medicine, Stanford University School of Medicine, CA.

Infantile neuroaxonal dystrophy (INAD; OMIM 256600) is a rare autosomal recessive neurodegenerative disorder characterized by psychomotor regression, visual impairment and dementia. Swollen axons (spheroids) are seen throughout the central and peripheral nervous systems. Neither the basic molecular defect nor the gene for this disorder has been identified.

A mutant mouse demonstrating early onset progressive neurologic impairment arose spontaneously in a congenic strain. In this colony, the incidence of affected offspring to known carrier parents is 25% with no gender bias, supporting an autosomal recessive pattern of inheritance. Although the pups appear normal at birth, affected mice are smaller than littermates, unthrifty and poorly groomed by 3-4 weeks of age. Beginning around 4 weeks, muscle wasting and weakness of the hind limbs becomes evident. In later stages of disease, the mice display blepharitis and paralysis of the hind limbs, generally requiring euthanasia by 6-8 months of age. Neuropathological studies reveal numerous axonal spheroids in both gray and white matter of the brain and through all levels of the spinal cord. The spheroids stain positively for PAS, with aggregates of mitochondria, increased neurofilaments, layered stacks of membranous loops and evidence for astrocytosis seen by light microscopy. Electron microscopy of degenerating axons demonstrates axoplasmic masses, degenerate organelles and membrane-bound dense bodies.

These mice may represent a model for INAD based on the pattern of disease inheritance and their early clinical and pathologic phenotype. Mammalian models for later onset or localized neuroaxonal dystrophies have been reported; however, none demonstrates the early and generalized phenotype seen in these mice. This novel mouse mutant may be valuable in identifying the human INAD gene and understanding the pathophysiology of neuroaxonal dystrophies.

Analysis of Spinocerebellar Ataxia Type 12 and DRPLA Genes In Colombian Individuals. *L. Perez¹, O.L Pedraza³, C. Duran¹, M.L Gomez¹, J.C. Prieto^{1,2}.* 1) Instituto de Genetica Humana, Universidad Javeriana, Bogota, Colombia; 2) Hospital la Victoria, Departamento de Genetica, Bogota, Colombia; 3) Departamento de Neurologia, Hospital San Ignacio, Universidad Javeriana, Bogota, Colombia.

Autosomal dominant cerebellar ataxias (ADCA) constitute a group of neurodegenerative disorders characterized by ataxia, dysphagia and dysarthria with onset of symptoms usually in the third or fourth decade of life that can be classified clinically into three major categories: ADCA type I is characterized by ataxia associated with other neurological features, ADCA type II is distinguished clinically by the presence of a pigmentary macular dystrophy and ADCA type III, which is characterized by a relatively pure cerebellar syndrome. Nine spinocerebellar ataxia genes (SCA1,2,3,6,7,8,10,12,17) and two other related ataxia genes (DRPLA, FRDA) have been cloned allowing the genetic classification of these disorders. Previously, we have reported that, among our ataxia patients, SCA8 occurs in 1.2%, FRDA in 28.9%, and Vitamin E deficiency in 3.6%. The symptoms and clinical signs observed in the patients were: gait and limb ataxia (100%), nystagmus (17%), pyramidal tract signs (21%), intellectual impairment (21%), epilepsy (7%), retinal degeneration (3%), and deafness (3%). We have now extended our testing to include DRPLA and SCA12. We investigated the normal size range of DRPLA and SCA12 alleles by genotyping normal Colombian individuals. CAG repeat ranges were 10-24 for DRPLA and 6-16 for SCA12. Frequencies of all alleles and heterozygosity were estimated and compared to several ethnic groups. We analyzed the CAG expansion of SCA12 and DRPLA in affected patients and did not find mutations in 108 patients studied. Variable percentages of known CAG repeat expansion have been reported in other ADCA studies. The ranges obtained for DRPLA and SCA12 in the Colombian population are below those reported for other ethnic groups. Thus, DRPLA and SCA12 do not appear to be an important etiological factor among our population. 70% of the patients studied did not have mutations in SCA1,2,3,6,7,8, DRPLA, or FRDA genes. Therefore, SCA10, SCA17, or other ataxia genes yet to be identified, might explain some of these cases.

Two novel mutations in the CACNA1A gene leading to identical functional consequences but with different phenotypic expression. *A.M.J.M. van den Maagdenberg¹, G.M. Terwindt², E.E. Kors², P. Imbrici³, N.J. Giffin⁴, C.R. Sherrington⁵, R.C.G. van de Ven¹, M.G. Hanna³, D.M. Kullmann³, P.J. Goadsby⁴, M.D. Ferrari², R.R. Frants¹.* 1) Department of Human Genetics, LUMC, Leiden, The Netherlands; 2) Department of Neurology, LUMC, Leiden, The Netherlands; 3) Department of Clinical Neurology, Institute of Neurology, University College London, Queen Square, London, United Kingdom; 4) National Hospital for Neurology and Neurosurgery, Queen Square, London, United Kingdom; 5) Department of Neurology, St. James University Hospital, Leeds, United Kingdom.

Mutations in the CACNA1A gene which encodes the pore-forming Cav2.1 subunit of neuronal P/Q type Ca²⁺⁺ channels have been shown to cause a spectrum of diseases, including familial hemiplegic migraine (FHM), episodic ataxia type 2 (EA-2), spinocerebellar ataxia type 6 (SCA6) and recently epilepsy. We screened all 47 coding exons of the CACNA1A/Cav2.1 gene by single-strand conformation polymorphism (SSCP) analysis and direct sequencing in DNA of the probands of two families. In one family a highly variable phenotype was observed, including migraine, fluctuating weakness, and ataxia, the second family showed typical EA-2 features. DNA analysis revealed two novel mutations in codon 1854 resulting in the same premature stop (Y1854X) in the predicted protein sequence. The truncation mutations are present in alternatively spliced exon 37A that is used mutually exclusively with exon 37B. Expression studies were performed in *Xenopus* oocytes using a CACNA1A construct harboring the Y1854X mutation and demonstrated no current, indicating a clear loss-of-function effect for this mutation. When co-expressed with the mutant, no effect was observed on wildtype Cav2.1 for several electrophysiological parameters. In contrast, previously described observations with nearby located truncation mutation R1820X, that is associated with epilepsy, showed a dominant-negative effect on wildtype. Apparently, multiple pathogenic mechanisms are associated with CACNA1A causing EA-2 and related phenotypes and mutations in the CACNA1A gene, even if they lead to the same functional defect, can cause a wide spectrum of clinical features.

A novel sequence motif highlights a functional link between spartin and spastin, mutated in hereditary spastic paraplegia. *H. Patel*¹, *F. Ciccarelli*², *C. Proukakis*¹, *M.A. Patton*¹, *P. Bork*², *A.H. Crosby*¹. 1) Dept Medical Genetics, St George's Hosp Medical Sch, London, England; 2) European Molecular Biology Laboratory, Meyerhofstr. 1, 69012 Heidelberg, Germany.

The hereditary spastic paraplegias (HSPs) are a clinically diverse group of disorders that share the primary feature of progressive lower limb spasticity and weakness. Despite the large and ever increasing number of loci mapped for the various forms of HSP, the genes for only a few forms of the disease have thus far been identified, each essentially with an unclear mechanism of action. We have recently shown that mutations in spartin underlie an autosomal recessive variant of HSP (Troyer syndrome) present at high frequency amongst the Amish. Comparative sequence alignment reveals that spartin contains three novel protein domains, one of which is also present in spastin, commonly mutated in autosomal dominant forms of HSP. This ~80 amino acid domain is located in the N-terminal region of spastin, which has recently been implicated in microtubule interaction. Multiple alignment confirms that this domain is also present in VPS4, SKD1 and SNX15, all of which are involved in endosomal trafficking. Consequently we have named this novel domain MIT (microtubule-associated domain involved in intracellular transport). The identification of the MIT domain in both spartin and spastin implicates a functional link and hints at a molecular mechanism responsible for these forms of HSP.

A large six generations Indian pedigree with congenital nystagmus: clinical and genetic data. *U. Radhakrishna¹, U.C Rao¹, U. Ratnamala¹, M. Raveendrababu¹, R. Singh², J.V. Solanki³ and Genetics Study group.* 1) GeneHealth, Green Cross Voluntary blood bank & Genetic Centre, 201, Anilkunj, Paldi, Ahmedabad; 2) Nayanjyot, Dr. P.L. Desai Eye Centre, Navrangpura, Ahmedabad; 3) Department of Animal Genetics & Breeding, Veterinary college, Gujarat Agriculture University, Anand, India.

Congenital nystagmus (CN) is characterised by bilateral uncontrollable ocular oscillations and it occurs at a frequency of 1/1,500 live births. Families with X-linked, autosomal dominant and autosomal recessive modes of inheritance have been reported. The genes responsible for X-linked (NYS1) and autosomal dominant (NYS2) CN have been mapped to chromosomes Xp11.4-p11.3 (*Am J Hum Genet* 64: 1141-46, 1999), Xq26-q27 (*Am J Hum Genet* 64: 600-7, 1999) and 6p12 (*Genomics* 33: 523-6, 1996), respectively, but no mutation causing gene is yet identified. We report a large six-generation Indian pedigree with isolated non-syndromic nystagmus (NYS2) (OMIM 164100), in which the anomaly segregates as an autosomal dominant trait. The pedigree consists of 87 individuals including 23 affecteds (15 males/8 females). Clinical examinations were carried out on nine selected affected individuals including colour vision examinations and eye movement recordings. All the examined individuals showed asymmetric pendular eye movements with unidirectional jerky nystagmus. The clinical status of the remaining family members were collected by history. No other associated anomalies such as decreased vision, strabismus, colour blindness, ocular albinism or congenital stationary night blindness were present in this family. Karyotype analysis of five affecteds showed no chromosomal anomaly. Linkage studies with markers closely linked to NYS2 will either confirm allelism to this locus or support evidence for genetic heterogeneity and may reduce the genetic interval encompassing the NYS2 gene.
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Recurrent mutations in PTCH1 gene observed in French patients with Gorlin syndrome. *P.P.E. Gorry^{1,3}, N. Boutet¹, D. Lafon¹, M.F. Avril², M. Longy¹, D. Lacombe³*. 1) Laboratoire d'Oncogenetique Institut Bergonie, Bordeaux, France; 2) Service de Dermatologie, Institut Gustave Roussy, Villejuif, France; 3) Service de Genetique Medicale, CHU Pellegrin-Enfants, Bordeaux, France.

We report novel, de novo and recurrent mutations in PTCH1 gene. Our results highlight the spectrum of mutations in Gorlin syndrome. While no phenotype-genotype correlation could be found, we discuss the criteria for clinical diagnosis in front of molecular studies. In conclusion, we have screened for PTCH mutations in French Gorlin patients for the first time. Half of our patient population was composed of sporadic cases. Novel mutations and polymorphisms were identified in this group of patients. While no mutations hot spot was previously described in the literature, we identified recurrent mutations. Beyond the high sensibility of dHPLC PTCH mutations screening, we could suspect that mutations of the promoter and intronic sequences, or small rearrangements of the gene, are probably under estimated. This should conduct us to develop complementary methods for PTCH molecular diagnosis. Finally based on this molecular study, we could propose that the hereditary character is not dispensable for the diagnosis of the Gorlin syndrome if dysmorphic symptoms are present.

Sex chromosomes escape non-homologous associations of interstrand cross-link-induced radial formation. *A.E.*

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Following exposure to high concentrations of DNA crosslinking agents such as mitomycin C (MMC) and diepoxybutane (DEB), mammalian cells display characteristic chromosomal abnormalities known as radials. Radials appear as exchanges between the chromatids in fixed mitotic cells that have been treated with DNA crosslinking agents. These figures presumably form due to a failed attempt to repair the resulting DNA interstrand crosslinks (ICLs) and had been thought to occur randomly between both homologous and non-homologous chromosomes. Cells from patients with Fanconi anemia (FA) display these same characteristic formations but with much lower concentrations of ICL-inducing agents. Although radial formation has been used to diagnose FA for more than three decades, there has been relatively little analysis and description of these chromosomal structures. Understanding the mechanism of formation of radials should help in elucidating the mechanism of ICL repair and the function of the FA pathway.

For this purpose, we analyzed G-banded radials from normal and FA primary human fibroblasts treated with appropriate concentrations of MMC. Among 382 radials analyzed, only three involved homologous chromosomes and these interactions were between different arms and/or regions of the chromosomes. In addition, the X and Y chromosomes were noticeably not involved in radial formation. Analysis of band location and association points revealed a random distribution of radials throughout the length of the autosomes, at both light and dark bands, with longer chromosomes being involved at an increased frequency, especially in FA cells. In addition, we found that radials can form between human and mouse chromosomes in hybrid cells. Together, these observations suggest that radials represent either failed recombination between short stretches of homologous sequences or simply random exchanges, primarily on autosomes. A model for ICL repair and escape of the sex chromosomes from radial formation will be discussed.

Identification of novel glomulin gene mutations responsible for inherited glomuvenous malformations

(glomangiomas). *P. Brouillard*¹, *M. Ghassibé*¹, *L.M. Boon*^{1,2}, *A. Penington*³, *O. Enjolras*⁴, *J.B. Mulliken*⁵, *M. Vikkula*¹.

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Glomuvenous malformations (GVMs, glomangiomas), localized defects of vascular morphogenesis, are single or multiple bluish-purple lesions that occur mainly in skin. Histologically, the distended veins present smooth muscle-like "glomus cells" in the media. GVMs are usually dominantly inherited and, with the more common mucocutaneous venous malformations, they are among the most frequent lesions in centers that specialize in treatment of vascular anomalies (Vikkula et al., 1998). Using positional cloning, we recently identified the causative gene that we named glomulin (Brouillard et al., *Am J Hum Genet* 2002). 13 of the 14 mutations identified in 20 families cause premature stop codons. Thus, GVMs are likely to be caused by loss-of-function of glomulin. In addition, as these inherited vascular lesions are localized, we hypothesized that a somatic second hit, leading to complete localized lack of glomulin, may be needed. In fact, we identified a truncating somatic mutation that was different from the patients inherited genetic alteration in a resected tissue sample. We report on the identification of a series of additional mutations, confirming the loss-of-function mechanism to be responsible for GVMs. (vikkula@bchm.ucl.ac.be) (<http://www.icp.ucl.ac.be/vikkula>).

Mutations in the genes *KCND2* and *KCND3* encoding the ion-channels conducting the cardiac transient outward current (I_{TO}) is not a frequent cause of Long QT syndrome. R. Frank-Hansen¹, L.A. Larsen², P.S. Andersen¹, C.

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Mutations in genes encoding cardiac potassium and sodium channel subunits are found in approximately 50% of patients diagnosed with long QT syndrome (LQTS), a potentially lethal cardiac arrhythmogenic disorder characterized by prolongation of the QT interval in electrocardiograms. The absence of mutations in the five known LQTS genes in the remaining half of the patients indicate the existence of additional genes associated with the disease.

The genes *KCND2* and *KCND3* encoding the potassium channels conducting the transient outward current (I_{TO}) in the cardiac action potential (AP) have been associated with prolongation of AP duration and QT prolongation in murine models. The role of *KCND2* and *KCND3* as candidates for LQTS association was investigated by mutation screening in DNA samples obtained from 43 unrelated LQTS patients, all without mutations in the five known LQTS genes. The mutation screening was performed by single strand conformation polymorphism, either gel-based or by automated capillary electrophoresis (CE-SSCP). The analysis revealed the existence of several single nucleotide polymorphisms (SNPs), both in *KCND2* and in *KCND3*. Based on mutation screening of 48 normal controls (danish blood donors), all SNPs are considered normal variants. The data suggests that mutations in *KCND2* and *KCND3* are not a frequent cause of long QT syndrome.

Screening of the *FBLN5* Gene to Identify Genetic Alterations in Patients with Aortic Tortuosity. *P.A. Gupta*¹, *R.M. He*¹, *D.G. Guo*¹, *S.N. Hasham*¹, *C.C. Miller*², *A.L. Estrera*², *H.J. Safi*², *D.M. Milewicz*¹. 1) Internal Medicine, UT Houston Medical School, Houston, TX; 2) Cardiothoracic Surgery, UT Houston Medical School, Houston, TX.

Fibulin 5, encoded by *FBLN5*, is a calcium-dependant, elastin-binding, extracellular matrix protein that is expressed in the cardiovascular system during embryogenesis and adulthood. The phenotype of the *FBLN5*-knockout mouse included severe tortuosity of the aorta, emphysema, and loose skin. Based on the murine phenotype, we screened the DNA of 22 unrelated patients with severe tortuosity of the aorta, termed megaaorta syndrome. We screened the 11 exons of *FBLN5* using genomic DNA from the patients, intron-based, exon-specific primers, and direct sequencing. Two polymorphisms were identified: C1407T in exon 9 and an A to G transversion 25 bp 5' to the start of exon 2. Two unique alterations were identified: an A to G transversion 29 bp 3' to exon 5 and a 1 bp deletion 238 bp 5' to the start codon in exon 1. The 1 bp deletion was not present in 120 ethnically matched chromosomes. The family members of both individuals with unique changes are currently being investigated. Further analysis of the two unreported alterations will provide information on the role that *FBLN5* plays in aortic disease.

Familial deafness, congenital heart defects, and posterior embryotoxon caused by cysteine substitution in the first EGF-like domain of JAG1. *C. Le Caignec¹, M. Lefevre², J.J. Schott³, A. Chaventre¹, M. Gayet⁴, C. Calais⁵, J.P.*

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Congenital heart disease is the most common birth defect, occurring in approximately 1% of live births. Although multifactorial inheritance has been postulated for the majority of cases, single gene transmission is demonstrated by the observation of mutations in NKX2.5 or JAG1 within families segregating congenital heart defects. We report a kindred with hearing loss, congenital heart defects, and posterior embryotoxon segregating as an autosomal dominant trait. Six of seven available affected patients manifested mild-to-severe combined hearing loss predominantly affecting middle frequencies. Two patients were diagnosed with vestibular pathology. All patients had congenital heart defects, including Tetralogy of Fallot (ToF), ventricular septal defect or isolated peripheral pulmonic stenosis (PPS). No individual in this family met diagnostic criteria for any previously described clinical syndrome. A candidate gene approach was undertaken and culminated in the identification of a novel Jagged1 (JAG1) missense mutation (C234Y) in the first cysteine of the first EGF-like repeat domain of the protein. JAG1 is a cell surface ligand in the Notch signaling pathway. Mutations in JAG1 have been identified in Alagille syndrome (AGS) patients. Our findings revealed a unique phenotype with highly penetrant deafness, posterior embryotoxon, and congenital heart defects but variable expressivity in a large kindred which demonstrate that mutation in JAG1 can cause hearing loss.

Hereditary Haemorrhagic Telangiectasia: ENG and ALK1 mutations in Dutch patients. *T.G.W. Letteboer¹, R.A. Zewald¹, J.J. Mager², C.J.J. Westermann², J.K. Ploos van Amstel¹.* 1) 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 clinical presentation can be quite heterogeneous. Specific organs tend to be more affected such as the skin, lung, gastrointestinal tract and the brain. The estimated prevalence in the Netherlands is 1:30,000, but this is very likely to be an underestimation. Mutations in at least two genes have been shown to be associated with the disease, endoglin (ENG on chromosome 9q34) and ALK1 (ACVRL1 on chromosome 12q13). We have studied 68 probands of Dutch origin with the Rendu-Osler-Weber disease performing sequence analysis on genomic DNA, for both the ENG gene (exon 1-14) and ALK1 gene (exon 2-10). To date, approximately 90% of the coding sequence has been analysed. Mutations were found in both genes: 43% of the probands had a mutation in the ENG gene, whereas 30% had a mutation in the ALK1 gene. No proband has been identified with mutations in both genes. The mutations detected were deletions, insertions, nonsense, missense and splice site mutations. Currently, we are organizing the clinical data to get insight in 1. the inter- and intra-familial variation of the phenotypes and 2. the genotype-phenotype relationship of the two patient groups with an endoglin respectively an ALK1 mutation. Appropriate medical attention can be given to those having a mutation, complications can be prevented and clinical screening of non-carrier children in the family becomes unnecessary.

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TBX5 is a transcription activator with a functional domain in its C-terminus. *T. Huang, L. Scott, L. Li, L. Feutch.*
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TBX5 is a member of the T-box gene family and encodes a transcription factor involved in cardiac and limb development. Mutations of TBX5 cause Holt-Oram syndrome (HOS), an autosomal dominant condition with congenital cardiac defects and forelimb anomalies. Here, we report that TBX5 is a transcription activator and that the functional domain is located in its C-terminus. Using a series of deletion mutations of TBX5, we narrowed down its functional domain to a cluster of amino acids. Point mutagenesis further elucidates the crucial amino acids. This result would enhance our understanding of the function of TBX5 and its structure-function relationship.

Identification of the gene responsible for familial capillary malformation. *M.S. Vikkula¹, I. Eerola¹, L.M. Boon^{1,2}, S. Watanabe³, J.B. Mulliken⁴.* 1) Laboratory of Human Molecular Genetics, Christian de Duve Institute of Cellular Pathology and Université catholique de Louvain, Brussels, Belgium; 2) Center for Vascular Anomalies, Clin univ St-Luc, Brussels, Belgium; 3) Division of Plastic Surgery, Showa University School of Medicine, Tokyo, Japan; 4) Vascular Anomalies Center, Children's Hospital, Harvard Medical School, Boston, MA, USA.

Vascular anomalies comprise a heterogeneous group of disorders the severity of which varies from life-threatening lesions to cosmetic harm (Vikkula et al., Trends in Cardiovascular Medicine, 1998 and Matrix Biology, 2001). They are localized defects of vasculogenesis/angiogenesis and thereby provide a tool to study the mechanisms involved in these processes. Capillary malformation (CM, or port-wine stain) is the most common vascular malformation occurring in 0,3% of newborns. CMs are small flat cutaneous lesions that consist of an increased number of ectatic capillary-like channels within papillary dermis. Vascular birthmarks, such as salmon patch, are milder variants of CM that occur in up to 40% of newborns. Unlike common macular stains, the reddish coloration of CMs does not disappear, but becomes darker with advancing age. Increased incidence of lesions in first-degree relatives of CM patients and several reported familial cases suggest that genetic factors may play a role in the pathogenesis of CM. Here we report on our genomewide linkage analysis performed on 13 families with inherited CM. In non-parametric linkage analysis, statistically significant evidence of linkage (peak Z-score 6.72, p-value 0.000136) was obtained in an interval of 69 cM on 5q11-5q23. Parametric linkage analysis gave a maximum combined HLOD score of 4.84 (a-value 0.67) from the same region and the analysis using only the linked families, defined a smaller, statistically significant locus of 23 cM (LOD score 7.22). Positional candidate gene analysis in this large region recently led to the identification of the mutated gene that seems to cause pathological angiogenesis by haploinsufficiency. Thus, a novel target for anti-angiogenic therapy has been unravelled.(vikkula@bchm.ucl.ac.be)(<http://www.icp.ucl.ac.be/vikkula>).

PTPN11 gene mutations in syndromic and non-syndromic pulmonary valve stenosis. A. Sarkozy^{1,2}, E. Conti¹, M.C. Digilio³, D. Seripa¹, B. Marino⁴, M.G. Matera¹, G. Esposito^{1,2}, V.M. Fazio¹, A. Pizzuti^{1,2}, B. Dallapiccola^{1,2}. 1) Dept. Exp. Med. and Pathol., Univ. "La Sapienza", Rome, Italy; 2) IRCCS-CSS, S. G. Rotondo and CSS-Mendel, Rome, Italy; 3) Division of Med. Genet., Bambino Gesù Hosp., IRCCS, Rome, Italy; 4) Sect. of Pediatric Cardiol., Inst. of Pediatrics, Univ. "La Sapienza", Rome, Italy.

Pulmonary valve stenosis (PVS), occurs in 4/10000 births. PVS can be isolated, or part of different syndromes. PVS is a common feature of Noonan (NS) and Multiple Lentiginos/LEOPARD (ML/LEOPARDS) syndromes. Both isolated and syndromic PVS are inherited as an autosomal dominant mutation. In syndromic PVS, the valvular stenosis is due to a valvular leaflet dysplasia, rather than to intrauterine hemodynamic factors, as probably in non-syndromic cases. However, syndromic PVS with normal leaflets has been also reported. Ptpn11 mutant mice show PVS with dysplastic leaflets, while PTPN11 mutations have been reported in NS and ML/LEOPARDS patients. In our cohort of 27 NS and ML/LEOPARDS patients with PVS, 41% had PTPN11 mutations. To investigate the role of PTPN11 mutations in the pathogenesis of different PVS, we screened PTPN11 gene mutations in 23 patients with non-syndromic PVS. PTPN11 coding region was screened by SSCP, and fragments with an aberrant migration were sequenced. The prevalence of PTPN11 mutations in syndromic patients with PVS was assessed both in those with normal or with dysplastic leaflets (20 vs. 7 patients). No patient with non-syndromic PVS presented PTPN11 mutations. Conversely, 73% of the 11 mutated syndromic patients with PVS, had leaflet dysplasia. PTPN11 mutations were detected in 40% of the PVS cases with dysplastic leaflets, and in 43% of those with normal leaflets. These results suggest a major relationship of PTPN11 mutations only with syndromic PVS, and not with non-syndromic cases. This conclusion is also supported by the higher incidence of PTPN11 mutations in syndromic PVS with valvular dysplasia. However, since three cases of syndromic PVS had normal leaflets, in association with PTPN11 mutations, occurrence of PTPN11 mutations in syndromic PVS should not be restricted to the presence of valvular dysplasia.

Carney Complex: Genetic analysis of the R1a Regulatory Subunit of Protein Kinase A (*PRKAR1a*). *M. Veugelers*¹, *Y. Song*¹, *J. He*¹, *B. Meyer*², *E. Legius*³, *D. Eccles*⁴, *A. Irvine*⁵, *C.T. Basson*¹. 1) Department of Medicine, Weill Weill Medical College of Cornell University, New York, NY; 2) Swiss Cardiovascular Center Bern, University Hospital Bern, Switzerland; 3) Center for Human Genetics, K.U.Leuven, Leuven, Belgium; 4) Wessex Clinical Genetics Service, Princess Ann Hospital, Southampton, UK; 5) Division of Dermatology, The Children's Memorial Hospital, Chicago, IL.

Carney complex is a familial multiple neoplasia syndrome characterized by cardiac myxomas in the setting of spotty skin pigmentation and endocrinopathy. Using positional cloning, we have previously identified the *PRKAR1a* gene (regulatory subunit R1a of protein kinase A) as a causative gene at chromosome 17q24. Analysis of the complete *PRKAR1a* coding region, by DNA-sequencing and dHPLC, was performed in 45 Carney complex probands, together representing 26 families and 19 apparently sporadic cases. We identified *PRKAR1a* mutations in 18 familial cases and 9 sporadic patients. Linkage to 17q24 and a potential locus at 2p16 could be excluded in 5 families. With the exception of two mutation hotspots in exon-5 and exon-7, all identified mutations were unique and randomly distributed across the coding region. There were no obvious genotype-phenotype correlations, but we observed the occasional presence of congenital heart disease, all in Carney complex patients with *PRKAR1a* mutations. RT-PCR analysis indicated that mutant mRNAs were degraded by nonsense mediated mRNA decay, causing *PRKAR1a* haploinsufficiency. However, three mutations escaped nonsense mediated mRNA decay. Western blotting analysis indicated that at least one of these mutations is expressed at the protein level. We observed increased cAMP-induced PKA activity in EBV-transformed lymphocytes of patients with *PRKAR1a* mutations. Our analyses indicate that most *PRKAR1a* mutations result in *PRKAR1a* haploinsufficiency and that in lymphocytes heterozygous for these mutations, there is an increased cAMP responsiveness causing dysregulated PKA activity. Furthermore, we have found evidence for genetic heterogeneity in Carney complex, and ongoing studies will identify novel genes that cause Carney complex.

Molecular analysis suggests the first sporadic case of FSHD in the South African population. *M. Alessandrini¹, A. van der Merwe¹, F.F. Maree^{1,2}, C-M. Schutte³, E. Honey⁴, R.R. Frants⁵, S.M. van der Maarel⁵, A. Olckers^{1,2}.* 1) Centre for Genome Research, Potchefstroom University, Pretoria, South Africa; 2) DNAbiotec, South Africa; 3) Dept of Neurology, University of Pretoria, South Africa; 4) Dept of Genetics, University of Pretoria, South Africa; 5) Dept of Human Genetics, Leiden University Medical Centre, The Netherlands.

Facioscapulohumeral muscular dystrophy (FSHD) is a progressive myopathy preferentially affecting the facial, shoulder girdle and upper arm muscles. It is one of the most common muscular dystrophies after Duchene and Myotonic dystrophy. Symptomatic presentation is generally observed in the second decade of life with a penetrance of approximately 95% by the age of 20 years. A high degree of variability is observed when comparing affected patients with signs of asymptomatic shoulder girdle or facial weakness in the 7th decade, to cases with severe proximal and distal upper and lower limb involvement in early childhood. FSHD follows an autosomal dominant mode of inheritance with a unique DNA rearrangement linked to the distal region of chromosome 4q35. However, no transcribed sequences in this region have been directly linked to the disorder. A deletion of an integral number of 3.3 kb repeat sequences at the 4q35 chromosomal region results in the clinical phenotype. Effective molecular diagnosis is, however, compromised due to several factors. The most striking of these being the simultaneous detection of a non-pathogenic region on chromosome 10q26, that not only displays a high degree of homology, but is also rearranged in a similar fashion to the 4q35 repeats. The frequency of sporadic FSHD cases has been reported to be 0.33 in the Brazilian population. Here we report the identification of the first sporadic case in the South African FSHD population. The patient was clinically diagnosed as severely affected at the age of 16 years. The presence of a 14kb deletion fragment was observed in this individual via Southern blot analysis using probe p13E-11. The parental status of the individual was confirmed via chromosome 4 haplotype analysis. The absence of deletion fragments in both of her parents confirms the sporadic nature of this case.

Genetic evidence for a possible novel SMA gene in a family with spinal muscular atrophy. *H.H. Lemmink, A.H. van der Hout, C. Lummen, J. Dijkhuis, H. Scheffer, C.H.C.M Buys.* Clinical Genetics, Academic Hospital Groningen, Groningen, Netherlands.

In around 95 percent of patients with spinal muscular atrophy (SMA) mutations have been identified in the SMN1 gene localized at chromosome 5q12-13. Recently, a second gene for SMA has been identified. Mutations were described in the immunoglobulin m binding protein 2 (IGHMBP2) gene localized on chromosome 11q13 in patients suffering from spinal muscular atrophy with respiratory distress (SMARD) resulting from paralysis of the diaphragm. This group of patients is clinically and genetically distinct from classical SMA patients (type I, II, and III) and probably represents around 1 percent of the total population of SMA patients.

We have studied two affected brothers who were clinically defined as classical SMA type I in accordance with examination of muscle biopsy specimen and EMG analysis. In this family we have excluded exon 7 deletions of the SMN1 gene and pathogenic mutations in IGHMBP2. Also linkage analysis was performed for both chromosomal regions (5q12 and 11q13) and both regions could be excluded. From our study we conclude that there is a possible third gene involved in the pathogenesis of SMA.

Generating mouse models for autosomal dominant hereditary Spastic Paraplegia Type 4 (SPG4). *J. Schickel¹, D. Boensch¹, R. Sudbrak², G.E. Homanics³, J. Neesen⁴, T. Deufel¹.* 1) Institut fuer Klinische Chemie und Laboratoriumsdiagnostik, Universitaetsklinikum Jena, Bachstr. 18, 07740 Jena, Germany; 2) Max-Planck-Institut fuer Molekulare Genetik, Ihnestr.73, 14195 Berlin, Germany; 3) Department of Anesthesiology and Critical Care Medicine, University of Pittsburgh, Pittsburgh, PA 15261, USA; 4) Institut fuer Humangenetik, Universitaet Goettingen, Heinrich-Dueker Weg 12, 37073 Goettingen, Germany.

Autosomal dominant hereditary spastic paraplegia (AD-HSP) is a genetically heterogeneous neurodegenerative disorder characterised by progressive weakness and spasticity of the lower limbs caused by the specific degeneration of the corticospinal tracts, the longest axons in humans. SPG4 is the most common form and is caused by mutations in the spastin gene, an ATPase belonging to the AAA family. The gene, located on chromosome 2p21-p22 is ubiquitously expressed in adult and fetal tissues. We have identified two SPG4 mutations in German AD-HSP families that result in measurably different phenotypes: A deletion of the first nucleotide of exon 9 leading to a premature stop codon is predicted to generate a truncated protein of 393 amino acids, and a large genomic deletion resulting in the loss of exons 13-16. Patients carrying those mutations differ clinically in transcranial magnetic stimulation studies providing a phenotypic correlate of a given genetic change in the SPG4 gene. With the aim to elucidate the function of the spastin gene and its role in the pathogenesis of spastic disorder, we have set out to generate genetically engineered mouse models carrying these two mutations defining distinct human disease phenotypes. We analysed the genomic organization of the mouse orthologue of SPG4 using screened PAC-clones. Subclones from the corresponding PACs were generated and further characterized by extensive restriction mapping and Southern blot analysis. On the basis of this work targeting vectors were designed and constructed. Electroporation of ES cells was performed and the identification for gene targeting is under way.

The first compound heterozygote individual identified in the South African FSHD population. *A. van der Merwe*¹, *M. Alessandrini*¹, *F.F. Maree*^{1,2}, *C.M. Shutte*³, *E. Honey*⁴, *R.R. Frants*⁵, *S.M. van der Maarel*⁵, *A. Olckers*^{1,2}. 1) Centre for Genome Research, Potchefstroom University for CHE, Pretoria, South Africa; 2) DNAbiotec, South Africa; 3) Dept of Neurology, University of Pretoria, South Africa; 4) Dept of Genetics, University of Pretoria, South Africa; 5) Dept of Human Genetics, Leiden University Medical Centre, The Netherlands.

Facioscapulohumeral muscular dystrophy (FSHD) is characterised by weakness and atrophy of the facial and shoulder girdle muscles. The phenotypical expression varies in severity, rate of progression and age of onset. The FSHD phenotype segregates as an autosomal dominant trait and is caused by a deletion of an integral number of 3.3kb repeat units on chromosome 4q35. However, the underlying genetic mechanism still remains elusive. Probe p13E-11 is utilised to detect the DNA rearrangements, but cross-hybridises to chromosome 10q26. Comparison between 4q35 and 10q26 indicated that both regions contain similar 3.3kb repeat units. The presence of a 24kb deletion fragment was observed in a South African FSHD family consisting of 8 generations and 480 individuals. Two deletion fragments of 24kb and 34kb, both in the pathogenic range, were detected in a single individual of this family. One of his three children inherited a 24kb fragment, while the other two inherited 34kb fragments. As it is known that translocation events may occur between chromosomes 4 and 10, the chromosomal origin of the 34kb fragment could not initially be determined with any certainty. Verification of the chromosomal origin of the 34kb fragment was, however, crucial for accurate molecular diagnosis of these two children. Haplotype analyses of chromosomes 4 and 10, utilising nine and six STRP markers respectively, were undertaken to confirm the chromosomal origin of this fragment. These analyses verified that the 34kb fragment is of chromosome 4 origin and therefore conclusively identified the first compound heterozygote case in the South African FSHD population. Correlation between severity of the disorder and the size of the deletion fragment within this family may be useful in the elucidation of the phenotype-genotype relationship in FSHD.

A dominant loss-of-phosphorylation IKBA mutation is associated with anhidrotic ectodermal dysplasia and severe T cell immunodeficiency. A.. Smahi¹, G. Courtois⁷, J. Reichenbach³, F. Le Deist², C. Cancrini⁶, R. Doffinger³, A. Durandy², M. Bonnet³, S. Dupuis-Girod⁵, M. Caniglia⁶, P. Rossi⁶, A. Fischer^{2, 4}, A.. Israel⁷, A. Munnich¹, J.L. Casanova^{3, 4}. 1) Genetique INSERM U393, Hopital Necker, Paris, France; 2) INSERM U429 Hopital Necker, Paris, France; 3) INSERM U550 Hopital Necker, Paris, France; 4) Unit d'Immuno-Hematologie, Pediatrique, Hopital Necker, Paris, France; 5) Hematologie Pediatrique, Hopital Debrousse, Lyon, France; 6) Division of Immunology and Infections Diseases, Bambino Gesù, Rome Italy; 7) URA CNRS 1773 Institut Pasteur, Paris, France.

An X-linked recessive form of Anhidrotic Ectodermal Dysplasia with Immuno-Deficiency (EDA-ID) has been ascribed to mutations in the NEMO gene, which encodes a regulatory subunit of the IKK complex, resulting in an impaired NF- κ B activation. We identified a boy with EDA-ID with no detectable NEMO mutation. In order to study the NF- κ B signalling pathway, we stimulated the patient's fibroblasts with TNF α and IL1 β . Electrophoretic mobility shift assay revealed that both stimuli failed to trigger NF- κ B DNA-binding into the nucleus. As the key step in NF- κ B activation is the phosphorylation of NF- κ B inhibitors, an IKK kinase assay was performed on patient's fibroblasts after TNF and IL1 stimulation. This test showed no defect in the IKK activation, but a strong and specific defect in I κ B α degradation. I κ B β and I κ B ϵ were degraded normally. It has been previously shown that the phosphorylation of two important residues of the I κ B α protein (S32 and S36) is crucial for its ubiquitination and degradation and the subsequent translocation of NF- κ B into the nucleus. For this reason, I κ B α cDNA and genomic DNA were completely sequenced and a *de novo* heterozygous G to T substitution was found (S32I). Remarkably, the immunological abnormalities of the EDA-ID syndrome caused by IKBA deficiency differ from those of the EDA-ID caused by NEMO mutations. Indeed, our IKBA-deficient patient had profound abnormalities of T lymphocyte development and proliferation. This is the first report of a mutation in the IKBA gene, the first evidence of its involvement in autosomal EDA-ID and its crucial role in T cell biology.

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PCR Technique and significance in Fragile X Syndrome. *L.A. Bastaki, SA. AL -AWADI, FA. HEGAZI, NA. TURKY, MA. MUSTAFA, KA. NAGUIB.* Maternity Hosp, Kuwait Medical Genetic Ctr, Khaldiya, Khaldia, Kuwait.

Objectives: To explore the potential application of PCR molecular methodology in detection of fragile X syndrome among suspected groups of mentally retarded male patients. Settings: Kuwait Medical Genetics Centre, Kuwait. Design prospective study included 182 mentally retarded males. Methods: 182 mentally retarded male patients who fulfill 5 or more criteria of fragile X syndrome were screened clinically and molecularly using the amplification of the triplet repeat sequences at FRAXA and FRAXE loci by PCR technique and southern blot in some positive cases. Results: 20 patients (10.9%) proved to be highly suspected of having fragile X syndrome due to mutation at FRAXA locus. None has mutation at FRAXE locus. Out of those 20 patients, 11 (55%) were confirmed to be positive by applying cytogenetic techniques while 7 cases (35%) were +ve by applying southern blotting techniques. Both prepubertal and post pubertal criteria of fragile X syndrome were discussed. Conclusion: The usage of PCR technique in diagnosis of fragile X syndrome is the most suitable technique in case of screening mentally retarded patients. Southern blotting technique is needed for confirmation of PCR positive results as well as detection of size repeat.

LMNA founder mutation in autosomal recessive axonal Charcot-Marie-Tooth disease reveals a novel

laminopathy. A. DeSandre-Giovannoli¹, M. Chaouch², S. Kozlov³, N. Kassouri², M. Tazir⁴, A. Vandenberghe⁵, T. Hammadouche⁶, C.L Stewart³, J.M. Vallat⁷, D. Grid⁸, N. Levy¹. 1) INSERM U491, Marseille, France; 2) service de neurologie, CHU Ben Aknoun, Algiers, Algrie; 3) Cancer and Developmental Biology Laboratory, NCI/Frederick, Frederick, MD; 4) service de neurologie, CHU Mustapha, Algiers, Algrie; 5) Laboratoire de Neurogenetique, Lyon, France; 6) Institut Pasteur, algiers, algrie; 7) Service de Neuropathologie, CHU Dupuytren, Limoges, France; 8) Genethon III, Evry, France.

Charcot-Marie-Tooth disease (CMT) is the most common motor and sensory inherited neuropathy, and, on the basis of electrophysiologic criteria, it has been divided into demyelinating (CMT1), and the axonal types (CMT2). Homozygosity mapping was performed on consanguineous algerian families affected with autosomal recessive (AR)-CMT2 and candidate genes from the critical interval were sequenced. In parallel, ultrastructural analysis from affected patients and Knock out mice were performed. Linkage to the 1q21..3 region was evidenced in 3 families. All affected individuals shared an ancestral homozygous haplotype. An homozygous LMNA (lamin A/C) mutation (C892T), causing an Arg to Cys substitution at the highly conserved residue 298 (R298C), was identified in all affected patients. A further analysis of CMT2 families from algeria identified the same homozygous mutation in patients from 5 more unrelated families. A sural biopsy from patient and nerves from Lmna ^{-/-} mice were examined. In human, a marked loss of myelinated and unmyelinated fibers was observed, with absence of large myelinated fibers. No onion bulbs or regenerating clusters were seen. Lesions of axons and myelin were also observed in nerves from Lmna ^{-/-} mice. Mutations in LMNA, encoding Lamin A/C, have been involved in Emery-Dreifuss myopathy, Dilated Cardiomyopathy, Lipodystrophy and, now, in a neuropathic phenotype (AR-CMT2). Deeper morphological and functional explorations on patients and Lmna KO and KI mice will give us new hints towards a better understanding of nerve-muscle interactions, and to the question as to whether mutations in the Lamin A/C proteins have tissue-specific or, viceversa, pleiotropic effects.

Multiple Mutations in PKHD1 in a Small Newfoundland Community. *L.C. Wilkins¹, E. Dicks², J. Green², P. Parfrey², W.S. Davidson¹.* 1) Molec Biol & Biochem, Simon Fraser Univ, Burnaby, BC, Canada; 2) Division of Medical Genetics, Memorial University of Newfoundland, St.John's, NF, Canada.

Newfoundland's geography, settlement, and socioeconomic development have produced a population useful for the study of genetic diseases. The presence of multiple genetic isolates and high coefficient of kinship have predisposed such isolates to autosomal recessive kidney diseases such as autosomal recessive polycystic kidney disease (ARPKD). ARPKD is a rare genetic disease with an estimated incidence of 1/20000 live births. It is characterized by enlarged and echogenic kidneys, as well as oligohydramnios secondary to a poor urine output. We have been examining a large extended kindred from a small Newfoundland community with six ARPKD affected individuals; three of which are of known consanguinity (Sibship A) and three of suspected consanguinity (Sibship B, C). Recently, the gene responsible for ARPKD has been identified. Mutational analysis was performed on the affected individuals of Sibship A and a homozygous mutation was identified (GLY-TRP, 11374 GGG-TGG). This same mutation was found in affected individuals of sibship B and C. Interestingly, the mutation in sibship B and C was heterozygous, indicating at least two mutations responsible for ARPKD in this small genetic isolate in Newfoundland. This observation agrees with haplotype analysis which predict two or more mutation carrying chromosomes associated with this disease. Other autosomal recessive diseases such as Bardet-Biedl Syndrome and Retinal dystrophy have also revealed multiple mutations in what was suspected to be a genetic isolate of a homogeneous origin in the Newfoundland population.

A t(4;6)(q12;p23) disrupts a novel gene, ADYC2, in a patient with acrodysostosis and short stature. *J.G. Dauwese¹, B.B.A. de Vries², C.H. Wouters², E. Bakker¹, G. Rappold³, M.H. Breuning¹, D.J.M. Peters¹.* 1) Center for Human and Clinical Genetics, Leiden University Medical Center, The Netherlands; 2) Clinical Genetics Center Rotterdam, The Netherlands; 3) Institute of Human Genetics, University of Heidelberg, Germany.

The index patient is a 34 year-old male, height 1.40m, arm span 1.26m, with normal intelligence and normal head circumference. He had extreme brachydactyly of both hands, more pronounced in the right hand, and to a lesser degree, brachydactyly of the feet. The right hand also shows partial syndactyly between digII and III. X-ray investigation showed acrodysostosis with relatively short ulna. In addition, the aorta was sclerotic with insufficiency of the aortic valves. Both parents had normal postures. A de novo 46,XY,t(4;6)(q12;p23) was found. BAC clones spanning the chromosome 4 and 6 breakpoints were identified by FISH. In close proximity of the chromosome 4 breakpoint the PDGFRA gene is located distally and the GSH2 homeodomain gene proximally. Both genes were not disrupted by the translocation. The GSH2 gene and functional parts of PDGFRA were screened for mutations in 150 individuals with idiopathic short stature. We only detected a stop mutation in the GSH2 homeodomain in two control individuals with normal postures, indicating that loss of one functional copy of GSH2 does not result in a disease-phenotype. The sequence of a chromosome 6 BAC clone revealed an EST close to the breakpoint. We sequenced the corresponding cDNA and identified a gene, named ADYC2 (acrodysostosis candidate 2), which is disrupted by the translocation in intron 1. ADYC2 is a novel gene with unknown function that spans 112Kb of genomic DNA. The gene consists of 13 exons, encodes a cDNA of 2783 bp with an ORF of 1487 bp and is ubiquitously expressed. The protein contains a MBOAT domain, which is found in proteins belonging to a superfamily of membrane-bound O-acyltransferases. In 150 persons with idiopathic short stature we did not find a mutation. In conclusion: We identified a novel gene, ADYC2, which is disrupted in a patient with acrodysostosis, but which is not commonly mutated individuals with short stature.

Myoclonus in a patient with a deletion of paternal e-sarcoglycan locus on chromosome 7p21. *R. Deberardinis¹, D. Conforto¹, K. Russell¹, J. Kaplan², B.S. Emanuel¹, E.H. Zackai¹.* 1) Division of Human Genetics and Molecular Biology, and; 2) Division of Neonatology, The Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA.

Autosomal dominant myoclonus dystonia syndrome (MDS) is characterized by myoclonic and/or dystonic movements in absence of dysmorphia, mental retardation, or growth abnormalities. In most families, MDS is caused by mutations in the gene SGCE, which encodes e-sarcoglycan and is located on chromosome 7q21. Data from several sources, including multi-generation pedigrees revealing parent-of-origin effects on MDS penetrance, suggest that SGCE is maternally imprinted. We present a 32-month-old patient with a complex phenotype including myoclonus, microcephaly, short stature, mildly dysmorphic facies and language delay. Chromosome analysis revealed an interstitial deletion affecting chromosome 7q21. We used fluorescence in situ hybridization to estimate the size of our patients deletion (9.0-15 Mbp) and to confirm absence of SGCE on the affected chromosome. Polymerase chain reaction analysis of polymorphic markers in the region revealed that the paternally inherited chromosome contained the deletion, consistent with a model of maternal SGCE imprinting. Our patient is the first case of MDS caused by a de novo interstitial deletion of the SGCE gene, and represents a new contiguous gene disorder. The case underscores the need to consider chromosomal deletions in patients whose phenotypes are more complex than the classic presentation of a known disease. Furthermore, the apparent maternal imprinting of SGCE and other genes on 7q21 raises the possibility that our patient, with a deletion on his paternally inherited chromosome, has a null phenotype for those genes. This mechanism, similar to that of Prader-Willi syndrome, should provide important information about the function of genes in the region.

Agenesis of the corpus callosum, severe mental retardation and intractible epilepsy in a female with a *de novo* paracentric inversion X(p11.2p22.1) that disrupts *ARX*. *Y. Peng, M. Kato, D.H. Ledbetter, W.B. Dobyns.* Dept of Human Genetics, University of Chicago, Chicago, IL.

Mutations of the X-linked gene *ARX* have recently been associated with a wide range of phenotypes in males including X-linked lissencephaly with abnormal genitalia (XLAG), X-linked infantile spasms (ISSX) or West syndrome, mental retardation with myoclonic epilepsy and spasticity, Partington syndrome (X-linked mental retardation and dystonia), and X-linked non-syndromic mental retardation. XLAG is associated with null mutations and missense mutations in the homeodomain (Kitamura et al., 2002; Kato et al., this conference), while the other phenotypes have been associated with missense mutations including a polyalanine expansion and a separate polyalanine duplication (Bienvenu et al., 2002; Stromme et al., 2002). Several mothers and sisters of genotypic males with XLAG have agenesis of the corpus callosum (ACC), associated with either a normal phenotype or mild mental retardation and rare seizures. No symptoms were mentioned in female relatives of boys with less severe *ARX* mutations. Thus, the spectrum of phenotypes in heterozygous females is not yet known. We report a girl evaluated at 10 months because of severe mental retardation and intractible epilepsy, including infantile spasms. Her brain MRI showed complete ACC with no other abnormalities. Chromosome analysis showed a small paracentric inversion: 46, X, inv(X)(p11.22p22.12) *de novo*. FISH on metaphase cells showed that PAC clone 258N20, which contains *ARX*, was split with part of the signal seen at each end of the inversion. Southern blots using *ARX* exon 5 as a probe showed abnormal bands with multiple enzymes. Analysis of the restriction map and results from Southern blot placed the telomeric breakpoint in a small 20 bp region in intron 4. X-inactivation studies using the androgen receptor assay showed a normal pattern (60:40 ratio). We conclude that *ARX* is disrupted in this patient, with truncation after exon 4. Her phenotype matches that of males with ISSX due to missense mutations of *ARX*. Thus mutations of *ARX* in females are associated with variable ACC, mental retardation and epilepsy.

A novel mutation in the protein C gene causing neonatal purpura fulminans. *K.K. Abu-Amero¹, M.H. Al-Hamed¹, F.S. Al-Batniji²*. 1) Dept. of Genetics, King Faisal Spcilaist Hospital, Riyadh, Saudi Arabia; 2) Pediatric Dept., Riyadh Armed Forces Hospital, Riyadh, Saudi Arabia.

Case report: A 9 months old Saudi boy was admitted to the NICU from the labor ward due to multiple bullae on the left lobar region of the abdomen, right cheek, right elbow and occipital region filled with fluid. The diagnosis of the epidermolysis bullosa was made and after the appearance of the scrotal haematoma, a haematology consultation raised the diagnosis of thrombosis due to protein C deficiency. He was treated with fresh frozen plasma (FFP), as PC-concentrate was not available. His parents are young, the father is 28 years old, while the mother is 25 yeras old and are first-degree relatives. Neither had personal or family history of thrombosis. The cousin of the index case had protein C deficiency diagnosed at another hospital, treated with fresh frozen plasma, but died at the age of one year. Methods: Genomic DNA was prepared from the patient and parents blood samples using standard methods. The 9 exons of the protein C gene were amplified by PCR using oligonucleotide primers described previously. Sequencing was carried out on the amplified PCR products using the same Primers used for amplification. Results: The index have severe protein C deficiency with PC activity level of < 0.01 U/ml (normal level 0.70-1.40 U/ml) and antigenic levels of 0.02 U/ml (normal level 0.70-1.40 U/ml). The father and the mother have PC activity levels of 0.69 and 0.66 U/ml respectively and Protein C antigenic levels of 0.67 and 0.63 U/ml respectively. Both parents and the index case were normal for Factor V Leiden mutation and Prothrombin G20210A variant. A previously undescribed mutation due to CCTG insertion after nucleotide 8826, in codon Thr-371 of exon 9 of the PC gene was found in the patient, in the homozygous state, and in both parents, in the heterozygous state. This insertion was not detected in a normal control. The mutation (8826, ins CCTG) resulted in a frame-shift with a stop at codon 381 resulting in premature protein termination. Conclusion: This frame-shift mutation agrees with the presence of quantitative PC deficiency in the affected member of the family.

Molecular mechanisms of Smith-Magenis syndrome - breakpoint analysis and gene identification. *W. Bi¹, J. Yan¹, P. Stankiewicz¹, S.-S. Park¹, K. Walz¹, L. Potocki^{1, 2, 3}, J.R. Lupski^{1, 2, 3}.* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Pediatrics, Baylor College of Medicine, Houston, TX; 3) Texas Children Hospital, Houston, TX.

Smith-Magenis syndrome (SMS) is a multiple congenital anomaly/mental retardation syndrome characterized by behavior problems, minor craniofacial and skeletal abnormalities, and sleep disturbance. More than 90% of SMS patients have an ~4 Mb heterozygous deletion of a genomic segment on chromosome 17p11.2 flanked by large and highly homologous low-copy repeats (SMS-REPs). Nonallelic homologous recombination between the SMS-REPs results in both the SMS deletion, and the reciprocal duplication of 17p11.2, a subtle clinical syndrome.

To determine the molecular aetiology of the apparent homology mediated recombination between misaligned SMS-REPs, we screened for the unequal crossing-over points in 14 patient somatic cell hybrids that retain only one allele of chromosome 17 with SMS common deletion. Our breakpoint analysis indicated that half of the patients have undergone a crossing-over event in an ~12 Kb region within the KER gene cluster. Thus, a recombination hotspot is located in the KER gene cluster within the ~170 Kb homologous regions between the SMS-REPs. Interestingly, there is an ~2.2 Kb AT-rich palindrome flanking the proximal KER gene cluster, which may potentially facilitate the unequal crossing-over.

To determine which genes are responsible for SMS through haploinsufficiency effects, we refined the critical region (SMCR) to an ~1.1 Mb genomic interval by breakpoint analysis of SMS patients with unusual deletions. Furthermore, preliminary studies suggest that the genes for SMS features may be located in an ~210 Kb interval within the SMCR on the basis of one individual with an unusual deletion who does have typical SMS feature. Eight genes are located within this region, including candidate genes *LLGL1*, *FLJ20308*, *SMCR7*, and *SMCR8*. The corresponding mouse genes *Flj20308*, *Smcr7*, and *Smcr8* have been assembled. To determine their roles in SMS, construction of mutated mice by gene targeting is in progress.

A novel mutation in PITX3 associated with an autosomal dominant form of congenital cataract. Z. Yang¹, W. Lin², G. Pellerano³, M.E. Valdez-Guerrero³, G. Pellerano-Nobo³, D. Taveras³, R. Caraballo³, L. Jiang¹, S. Thirumalaichary¹, J. Pan¹, K. Zhang¹. 1) Moran Eye Center and Program in Human Molecular Biology & Genetics, University of Utah, Salt lake city, UT; 2) Division of Health Sciences and Technology, Harvard Medical School, Boston, MA; 3) Complejo Hospitalario Dr.Luis E. Aybar,Santo Domingo, Dominican Republic.

Purpose: To report clinical phenotype and genetic study in one large family with congenital cataract. **Methods:** Ophthalmic examination and genetic study were conducted in one large kindred with an autosomal dominant form of congenital cataract. Genomic DNA was extracted from blood samples of members of this kindred. Genotyping analysis was performed using polymorphic DNA markers encompassing Paired-like Homeodomain Transcription (PITX3) gene. Each of the 4 exons of PITX3 gene was amplified by PCR and sequenced using Beckman CEQ2000 sequencer. **Results:** Of 25 individuals who were at risk of inheriting the disease gene in one family with an autosomal dominant form of congenital cataract, 15 were diagnosed with congenital cataract based on decreased visual acuity and ophthalmic examination. The onset of disease phenotype is at birth. The disease phenotype progresses from nuclear sclerosis to total cataract. Linkage analysis with short tandem repeat polymorphic markers revealed positive linkage to 10q24-25 with a lod score of 4.4 at theta=0 with D10S1239. DNA sequence analysis identified a 1-bp deletion (550delG), leading to a frame-shift, losing 86 amino acid fragment at the C-terminus, and synthesis of an aberrant peptide from amino acid position 217 to 307. This sequence change segregated with the disease phenotype and was not observed in 200 normal controls. **Conclusions:** Congenital cataract in this family is caused by a novel PITX3 gene mutation. Mutations in PITX3 have been reported in anterior segment mesenchymal dysgenesis and cataract. This study confirms that the PITX3 gene play an important role in normal eye develop and lens formation.

The MFS2 gene, the second gene implicated in Marfan syndrome, is now located in a region of 500KB. *G. Collod-Beroud¹, K. Dahan¹, M-C. Bouterin¹, M. Mouiseddine¹, G. Jondeau², G. Delorme², O. Dubourg², J-P. Bourdarias², C. Junien^{1,3}, C. Boileau^{1,3}.* 1) INSERM U383, Hopital Necker-Enfants Malades, AP-HP, Paris, France; 2) Service de Cardiologie, Hopital Ambroise Pare, AP-HP, Boulogne, France; 3) Laboratoire Central de Biochimie, d'Hormonologie et de Genetique Moleculaire, Hopital Ambroise Pare, AP-HP, Boulogne, France.

MFS was the founding member of the "heritable disorders of connective tissue". Cardinal manifestations of this autosomal dominant syndrome involves the eye, skeleton, and cardiovascular systems. It is now well established that defects in the fibrillin gene located on chromosome 15 (FBN1) cause not only classic Marfan Syndrome (MFS), but also a large range of milder, overlapping phenotypes. By excluding the FBN1 locus in a large French family we raised the issue of genetic heterogeneity in MFS and the implication of a second locus (MFS2). Linkage analyses, performed in this family with dispersed anonymous DNA markers, localized MFS2 initially to a region of 9 cM between D3S1293 and D3S1283 which map at 3p24.2-p25. The analysis was subsequently enlarged to new family members and new dinucleotide and tetranucleotide DNA markers. These data compiled with our data of positionnal cloning allowed us to order markers and to refine the genetic localization of MFS2 between markers D3S2336 and D3S2466. This region has an estimated physical distance of nearly 0.5 Mb. The transcriptionnal map of the critical region contains only 2 known genes that are poor candidates (RAR beta, and TOP2B) and 2 mRNAs coding for human homologs of genes known in other species. We are currently studying these two candidate genes. The expression of these candidates observed in a range of 13 different tissus is consistent with the features observed in MFS. Large and small rearrangements, point mutations as well as qualitative or quantitative transcript abnormalities are under investigation to determine which of these two genes is the MFS2 gene.

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De novo mutation of the connexin 26 gene associated with recessive non syndromic deafness. *Y. Hujeirat, S. Shalev.* Genetic Institute, HaEmek medical center, Afula, Israel.

Mutations of the connexin 26 (GJB2) gene account for about 50% of all genetic sensorineural hearing loss. We report a 18 months old Arab moslem boy who has congenital hearing loss. He is the first child of healthy young parents, who are not related. Molecular tests for 35delG, W77R, R184P, 101delAG and V37I mutations which have been reported among Arabs in the region revealed that the proband has 35delG and R184P mutations. The father is a carrier of 35delG, and the mother was negative for both mutations. Segregation analysis of polymorphic markers (from chromosome 7,9,11,13,17)confirmed the presence of biparental contribution. This is the first de novo mutation of connexin 26 associated with recessive hearing loss.

IS THERE AN ASSOCIATION BETWEEN DELETIONS/POINT MUTATIONS IN THE DYSTROPHIN GENE AND MENTAL IMPAIRMENT IN DUCHENNE/BECKER PATIENTS ? *D.N.F. Vagenas^{1,2}, A.M.P*

Cerqueira¹, A. Torres¹, R.C. Pavanello¹, M.R. Passos-Bueno¹, M. Zatz¹. 1) Centro de Estudo Genome Hum, Univ de São Paulo, Sao Paulo, Brasil; 2) Centro em interunidades em biotecnologia, USP, São Paulo, Brasil.

Duchenne (DMD) and Becker (BMD) muscular dystrophies are allelic X-linked recessive disorders caused by mutations in a gigantic gene, with 79 exons, which encodes dystrophin. The phenotype is caused in about 60% of the cases by deletions, 5-6% by duplications, while the remaining cases are caused by point mutations. About 30-50% of the DMD patients have mental impairment (MI) but its cause is still unknown. An association between MI and deletions/mutations in the region encompassing exons 45-79 exons, involved in brain dystrophin isoforms has been suggested. In an attempt to confirm this observation we are assessing the mental ability (based on the intelligence Scale for Children/WISC) in a very large group of DMD/BMD patients. Among 1092 patients who were ascertained in our center, 692 (analyzed through multiplex PCR, SSCP, PTT and sequencing) had the diagnosis of dystrophinopathy confirmed: 654 have deletions and 38 point mutations. Mental capacity evaluated in 519 patients showed that 239 (46%) have MI while the remaining 54% have an apparently normal IQ. When the analysis was repeated for different regions of the gene, we observed that the proportion of patients with MI was 39% (50/127) for the proximal region (exons 1-20), 47% (178/377) for the central region (exons 41-54) and 46,9 % (164/349) for the region encompassing exons 45 to 79. Understanding the cause of MI in Duchenne dystrophy remains a great challenge. Supported by CEPID-FAPESP, PRONEX, CNPq.

More than 500 FBN1 mutations associated with Marfan syndrome and overlapping disorders in the extensive update of the FBN1 mutation database. *S. Le Bourdelles¹, I. Dehaupas¹, S. Grey¹, C. Junien^{1,2}, C. Beroud^{1,3}, C. Boileau^{1,2}, G. Collod-Beroud².* 1) Laboratoire Central de Biochimie, d'Hormonologie et de Genetique Moleculaire, Hopital Ambroise Pare, AP-HP, Boulogne, France; 2) INSERM U383, Hopital Necker-Enfants Malades, AP-HP, Paris, France; 3) Laboratoire de Biochimie et Biologie Moleculaire, Hopital Cochin, AP-HP, Paris, France.

Marfan syndrome (MFS) is a dominantly inherited disease of connective tissue involving the eye, skeleton and cardiovascular systems. Mutations in the FBN1 gene (encoding fibrillin-1, the major structural component of 10-12 nm microfibrils of the extracellular matrix) were first associated with MFS and subsequently with a large spectrum of conditions phenotypically related to this disorder like ectopia lentis, neonatal MFS, isolated skeletal features (or Marfanoid skeletal syndrome), atypical MFS without cardiac implication, some cases of thoracic aortic aneurysms... All the mutations described in FBN1 are widely distributed throughout the gene with no apparent disease specific or structural/function relationship. To date, the only genotype/phenotype relationship is still limited to exons 24 to 32 in which most mutations causing neonatal MFS are clustered. To facilitate the mutational analysis of FBN1 mutations and the identification of structure/function and phenotype/genotype relationships, we created, in 1995, a human FBN1 mutation database which gives access to a software package that provides specific routines that are optimized multicriteria research and sorting tools. The database has been modified to follow the guidelines on mutation databases of the Hugo Mutation Database Initiative. The current update shows more than 500 FBN1 mutations. The global molecular analysis of the mutations file reveals that percentage of recurrence is more important (25%) than initially suspected; number of de novo mutations compared to transmitted mutations is surprising (48.5%); One third of the mutations are predicted to result in truncated proteins; Two third of the mutations are missense mutations affecting primarily the numerous calcium binding EGF-like modules. The database is available at <http://www.umd.necker.fr/>.

Molecular analysis of 840 mutations in the human LDL receptor gene database (UMD-LDLR). *J. Rabes^{1, 2}, L. Villéger¹, M. Abifadel^{1, 3}, D. Allard¹, C. Béroud¹, C. Junien^{1, 2}, C. Boileau^{1, 2}, M. Varret¹.* 1) INSERM U383, Paris, France; 2) Service de Biochimie, CHU Ambroise Paré, Boulogne, France; 3) Faculté de Pharmacie, Université Saint-Joseph, Beirut, Lebanon.

To date, more than 900 mutations have been identified in the LDLR gene, encoding the low-density lipoprotein receptor, in subjects with Familial Hypercholesterolemia. We have compiled a database containing standardized data for each LDLR mutation, and developed the software that provides sorting tools and allows optimized multicriteria research [<http://www.umd.necker.fr:2004>]. The analysis of the 840 point mutations in the UMD-LDLR database gives the following information: [1] 60% of the mutations are missense, and 21% occur in CpG dinucleotides known to be mutational hot spots; [2] although widely distributed throughout the gene, there is an excess of mutations in exons 4 ($P<0.001$) and 6 ($P<0.05$)(ligand-binding repeats), 7 ($P<0.05$) and 9 ($P<0.001$)(EGF-like repeats); [3] there is a deficit of mutations in exons 10 ($P<0.02$) and 13 ($P<0.001$)(EGF-precursor-like), 15 ($P<0.001$) (O-linked-sugar), 16 ($P<0.001$) (transmembrane), 17 ($P<0.02$) and 18 ($P<0.01$)(cytoplasmic); [4] 41% of the small deletions occur between repeated sequences and can be explained by the slipped-mispairing model described by Krawczak and Cooper; [5] 66% of the mutations in the ligand-binding domain affect conserved amino-acids involved in LDL binding; [6] the functional data available for 183 (29%) mutations indicate 38% of class 2B (transport defective) and 33% of class 1 mutations (null alleles); [7] finally, the investigation of genotype/phenotype correlations is difficult since the clinical data is usually incomplete in mutation reports.

Clinical and molecular variants of ceroid lipofuscinoses among French patients. *C. Caillaud, J.P. Puech, J. Dussau, L. Poenaru.* Dept Genetique, Cochin Institute, Paris, France.

Neuronal ceroid lipofuscinoses (NCL) are inherited neurodegenerative disorders characterized by the accumulation of autofluorescent lipopigments in various tissues. Their clinical signs are psychomotor retardation, impaired vision, seizures and premature death. Four main NCL forms are distinguished according to clinical and morphological features : infantile (INCL), late-infantile (LINCL), juvenile (JNCL) and adult (ANCL), but numerous atypical forms have also been described. Eight loci have been delineated as responsible for these different forms (from CLN1 to CLN8), some of them encoding soluble enzymes : palmitoyl protein thioesterase for CLN1, tripeptidyl-peptidase I (TPP-I) for CLN2.

LINCL is the most common clinical form in France. The CLN2 locus was frequently involved, as demonstrated by the presence of a tripeptidyl-peptidase I deficiency in a majority of patients. Complete sequencing of the CLN2 exons showed the predominance of two previously reported mutations (Arg208Stop and 3556 G>C), accounting each for around 30 % of the alleles. Novel private mutations were also characterized, such as deletions, splice, nonsense or missense mutations. A TPP-I deficiency was also found in one patient with JNCL carrying the 3556 G>C mutation in association with a novel point mutation. Some LINCL patients had no TPP-I deficiency and are likely candidate for the other NCL loci. Further studies will be focused first on the recently cloned CLN6 gene and later on the CLN7 locus, when characterized.

Classical INCL patients exhibited palmitoyl-protein thioesterase (PPT) deficiency and common or rare mutations in the CLN1 gene. Involvement of this gene was also demonstrated in one patient with an early juvenile form. Classical JNCL forms were associated either with the common 1.0 kb deletion or with private mutations of the CLN3 gene. Our results will allow to delineate the NCL loci involved in variant forms of NCL and to offer a reliable genetic counselling and prenatal diagnosis to couples at-risk for these diseases.

Enigmatic complexity in neurofibromatosis type 1 (NF1): three independent pathological mutations in the *NF1* Gene in an NF1 family. *M. Upadhyaya*¹, *E. Majounie*¹, *P. Thompson*¹, *S. Han*¹, *C. Consoli*¹, *I. Cordeiro*², *D. Cooper*¹.
1) Dept Medical Genetics, Univ Wales Col Medicine, Cardiff, Wales; 2) Uniclade de Genetica, Pediatria-P1808, Lisbon, Portugal.

Neurofibromatosis type 1 (NF1) is one of the most common autosomal dominant disorders with a prevalence of ~1 in 3500. The mutation rate calculated for the *NF1* gene ($3.1-10.4 \times 10^{-5}$) is one of the highest reported in humans. About half of all NF1 cases represent new mutations. The identification of inherited lesions in the *NF1* gene has proven difficult owing to the size and complexity of the gene, the existence of numerous processed pseudogenes and the considerable allelic heterogeneity manifested by NF1 patients. We report the identification of 3 independent pathological mutations of the *NF1* gene in a Portuguese NF1 family. Of two affected sibs, one had a 1.5 Mb maternally derived deletion whilst the other possessed a paternally derived nonsense mutation in exon 22 (R1241X). Both parents were clinically normal. A paternal cousin, was subsequently also diagnosed and was found to harbor a novel disease causing frameshift mutation in exon 29 (5406insT). This change was not present in either of his parents. Haplotyping analysis excluded both non-paternity and genetic recombination in all three patients. The disease allele-bearing chromosomes from the two sibs were different from the disease allele-bearing chromosome of their cousin. No obvious microsatellite alterations were detected in any member of the family suggesting that genome-wide instability was not an explanation for these findings. All three lesions occurred *de novo* on different chromosomal backgrounds and are likely to have been generated by radically different mechanisms viz. recombination, methylation-mediated deamination and slipped mispairing respectively. No evidence for mosaicism was identified in the parents. The probability of finding an NF1 family with two additional independent non-identical, pathological *NF1* gene lesions occurring *de novo* must be of the order of $(10^{-4})^2 = 10^{-8}$.

Using *Drosophila melanogaster* to identify Angelman syndrome therapeutic targets. *L.T. Reiter, M. Bowers, E. Bier.* Dept. of Biology, UCSD, La Jolla, CA.

The human *UBE3A* gene encodes an E3 type ubiquitin ligase, E6-AP, that marks specific protein targets for ubiquitin-mediated degradation. *UBE3A* is expressed biallelically in most human tissues, but is genetically imprinted in the brains of mammals. An overabundance of the proteins targeted by functional E6-AP in hippocampal and cerebellar neurons is potentially the cause of the neurological defects observed in AS patients. Identifying these target proteins could lead to new therapeutic strategies. Using the [Homophila](#) database, we identified a *Drosophila* homologue of *UBE3A*, which we named the *Drosophila* Angelman syndrome gene (*d-as*). Two different transcripts of *d-as* were cloned from whole flies by RT-PCR. The shorter transcript uses a cryptic splice site in exon V fused to exon I resulting in a protein product that is 71% similar to human E6-AP. The larger transcript is expressed in a developmentally regulated fashion and in the adult head. Using antibodies against human E6-AP distinct protein isoforms were identified in embryos and adult heads. Antibody staining indicates expression of D-as protein in the early CNS and endocrine secreting cells of the embryonic proventriculus. Using an existing strain containing a p-element inserted into the 5-UTR of *d-as*, we generated both lethal and semi-lethal alleles of *d-as*. Heterozygotes carrying a putatively downregulated allele over a lethal overexpressing allele exhibit a progressive degenerative phenotype in the eye. Ubiquitous temperature induced misexpression of human E6-AP or D-as results in neurologically compromised flies and death after two hours. With these reagents in hand we have initiated a genetic screen for second site modifiers of *d-as* mutant phenotypes to identify potential protein targets of D-as. We are also performing 2-D gel electrophoretic analysis on protein extracts from D-as misexpressing flies to identify proteins which have been downregulated by excess E6-AP or D-as. Once the D-as protein targets have been isolated, we will search for the corresponding proteins in humans and assay their abundance in the brains of AS patients as well as the corresponding mouse model for AS.

***RMRP* Gene Mutations and Clinical Presentation of Cartilage-Hair Hypoplasia: Extensive Allelic Heterogeneity and Lack of Genotype-Phenotype Correlations Indicate Modifier Genes.** A. Superti-Furga^{1,7,8}, A. Atkinson², E. Kirk³, S. Unger², M. DiRocco⁴, R. Savarirayan³, G. Mortier^{5,8}, B. Zabel^{6,8}, J. Ziegler³, C. Roifman², L. Bonafé^{1,7,8}. 1) Univ Childr Hosp, Zurich, Switzerland; 2) Hosp Sick Children, Toronto, Canada; 3) Sydney Childr Hosp, Sydney, Australia; 4) Ist Gaslini, Genova, Italy; 5) Med Genet Centre, Gent, Belgium; 6) Univ Childr Hosp, Mainz, Germany; 7) Univ Childr Hosp, Lausanne, Switzerland; 8) European Skeletal Dysplasia Network.

To characterize the clinical spectrum associated with mutations in the *RMRP* gene responsible for Cartilage-Hair Hypoplasia (CHH; MIM 250250), we have performed mutation analysis in patients with classical presentation (skeletal dysplasia and immune deficiency) as well as with partial phenotypes such as isolated metaphyseal dysplasia (MIM 250460), or immune deficiency and/or anemia in infants and young children without recognizable metaphyseal changes. We have identified four reported and thirteen unreported mutations in ten unrelated non-Finnish families. All probands are compound heterozygous, with no instance of homozygosity. Mutation types include insertions between TATA box and transcription start as well as single nucleotide substitutions at base-pairing and non-pairing residues. No relationship emerges between type of clinical presentation and type of mutation. - **Conclusions:** (1) extensive mutation heterogeneity and abundance of polymorphism suggest high mutability of the *RMRP* gene; (2) broad phenotypic spectrum and lack of strong genotype-phenotype correlations point to the existence of disease-modifying genes; (3) clinical presentations may include immuno-hematologic disease before metaphyseal dysplasia can be recognized; as such children may profit from timely therapeutic interventions, early molecular diagnostics can be important.

Analysis of *SFTPB* in 40 Sporadic or Familial Neonatal and Pediatric Cases with Severe Unexplained

Respiratory Distress. *M. Tredano*¹, *M. Griese*², *J. de Blic*¹, *T. Lorant*¹, *C. Houdayer*¹, *F. Cartault*³, *J. Elion*¹, *R.*

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We have analyzed the surfactant SP-B protein and its encoding gene (*SFTPB*, MIM 178640) in 40 unrelated pediatric patients with unexplained respiratory distress (URD). There was high consanguinity (8 kindreds) and an underlying autosomal recessive trait could be inferred in most cases, with overall high sex ratio (32/17) suggesting proband's gender to impact on penetrance. The clinical/biological presentations fitted into three major nosologic frames. I, SP-B deficiency (9 probands, scarcer than previously suggested), complete or incomplete, with homozygous/compoundly heterozygous mutations identified (6 probands), including one from the population isolate of Réunion Island (496delG). In addition, there was a consanguineous kindred in which incomplete deficiency was unambiguously unlinked to *SFTPB*. II, Pulmonary alveolar proteinosis (PAP, 19 probands), with typical storage of PAS-positive material within the alveoli with foamy macrophages and variable interstitial reaction, which was diagnosed in most patients from Réunion Island. Mutation and/or segregation analyses excluded *SFTPB* as a disease locus, although slight metabolic derangement related to SP-B and/or mild *SFTPB* changes could somehow contribute to disease. III, URD without evidence for SP-B deficiency or PAP (12 probands), equally unlinked to *SFTPB*, although a singular patient had a unique, possibly causal, maternally-derived heterozygous genetic change (G4521A). The population frequency of five known and four novel SNPs was studied, providing as many potential markers for pulmonary disease related to *SFTPB*. Overall, URD was found to be heterogeneous, both phenotypically and genetically, even in population isolates where founder effect might have been expected.

A homozygous cathepsin C gene mutation is responsible for Haim-Munk syndrome. *L.A. De Marco¹, R.S. Gomez², J.E. Costa², V.F. Cury¹.* 1) Dept Pharmacology, Univ Federal de Minas Gerais, Belo Horizonte MG, Brazil; 2) Department of Oral Surgery and Pathology, Univ Federal de Minas Gerais, Belo Horizonte MG, Brazil.

Palmoplantar keratoderma is a heterogeneous condition characterized by hyperkeratosis and erythema of the soles of the feet and palms of the hands. Haim-Munk syndrome (HMS) (MIM # 245010) and Papillon-Lefevre syndrome (PALS) (MIM # 245000) are rare autosomal recessive type IV palmoplantar ectodermal dysplasia and differs from other palmoplantar keratosis by their association with severe early onset periodontitis. Haim-Munk syndrome is a disorder that involves hyperkeratosis, arachnodactyly and nail deformities (onychogryphosis). The skin manifestations and nail deformities in HMS are more extensive and severe and the periodontium is less severely affected, in contrast to PALS. Several mutations of the lysosomal protease cathepsin C gene have been described in Papillon-Lefevre syndrome and only two mutations have been associated with Haim Munk syndrome. However, genotypic and phenotypic correlation has not been completely established. In the present study we describe a subject affected with Haim-Munk syndrome. DNA sequence analysis of CTSC gene from the affected patient identified one mutation (587TC) in exon 4, that altered the original CTT to a CCT codon. This mutation results in the substitution of a conserved leucine residue at position 196 by a proline (Leu196Pro). Of the 40 controls tested, none was found to have this missense mutation. This same mutation has been previously found to cause Papillon-Lefevre syndrome in one unrelated Brazilian kindred. Our findings suggest that the 587TC mutation may have either been inherited from common ancestors or other genetic or environmental factors could be important determinants of the clinical phenotype of both conditions. However, given their ethnicity, it is highly unlikely that there is a common ancestor. In summary, we here show a novel mutation in the cathepsin C gene responsible for Haim-Munk syndrome, suggesting that both Haim-Munk and Papillon-Lefevre syndromes are allelic variants of cathepsin C gene mutations, and other factors may be important determinants of the clinical phenotype.

Keratin-9 gene mutation in a family with EPPK. *L. Peleg*¹, *M. Karpati*¹, *B. Goldman*¹, *B. Amichai*². 1) The Ganek Gertner Institution of Human Genetic' Sheba Medical Ctr, Ramat Gan, Israel; 2) Department of Dermatology, Huzot Clinic of Kupat Holim, Ashkelon Iarael.

Epidermolytic palmoplantar keratoderma (EPPK), Voerners type is an autosomal dominant disorder, which belongs to a heterogeneous group of genodermatoses. The gene associated with EPPK was mapped to 17q11-q23, to the type 1 (acidic) keratin gene cluster. In 1993 the cDNA of Keratin 9 (KRT9) was cloned and sequenced. The gene product is expressed in the epidermis of the palms and soles. Most of the mutations associated to EPPK have been located within the highly conserved domain of the first α -helix motif of the KRT9 polypeptide (residues 156-171). A mutation in a different region was reported in one family only. The known mutations have been identified in varying ethnic background. We studied four affected and two non-affected individuals of three generations of an Ashkenazi Jewish family from Russia. In addition DNA samples of two non-related healthy controls were also analyzed. An A to G substitution at position 545 was identified by sequencing a 200 bp fragment (bp 497-697). The mutation generate a substitution of asparagine to serine at position 160. Since this mutation does not alter a recognition site of any known restriction enzyme, its presence was confirmed by sequencing all the studied DNA samples. The mutation was restricted to the affected individuals' DNA and was not found in the four healthy individuals. As this mutation was already reported in an Austrian-Alpine family with EPPK, an analysis of the polymorphic CA repeat region in intron 4 of the gene was undertaken. In the Austrian family the disease was associated with an allele containing 21 repeats. In the present family (six members) four alleles were revealed: 17, 20, 22 and 23 CA repeats. The mutation 545A to G was associated with the allele containing 20 repeats. These results suggested that different ancestors introduced the mutation in the two families.

Facioscapulohumeral dystrophy: Premature activation of the myogenic program? *D.A. Figlewicz, J.E. Sowden, A. Haefele, J.R. Forrester, K. Barrett, V. Kavcic, R. Tawil.* Dept Neurology, Univ Rochester Medical Ctr, Rochester, NY.

Facioscapulohumeral dystrophy (FSHD) is an autosomal dominant disorder resulting from deletion of 3.3kb repeats on chr 4q35. Part of the phenotype of the FSHD deletion(s) is observed in myoblasts prepared from FSHD patient muscle biopsy. Our previous studies suggest that FSHD myoblasts may be dividing less robustly either because they are leaving the cell cycle early, or because they are displaying replicative senescence. The cyclin dependent kinase inhibitor, p21, is upregulated in FSHD myoblasts; it is important to know whether this occurs in conjunction with the transcription factor MyoD (as observed in normal muscle differentiation), or whether it occurs independently of MyoD. Our recent data support the former option. FSHD myocytes fuse at a faster rate than controls, suggesting that the muscle differentiation program has been turned on. Quantitation of actual mRNA levels of MyoD and 4q35 genes which are expressed in muscle (ANT,ALP/SMT7,hFAT) confirms their highly significant upregulation. Moreover, Pearson Correlation analysis reveals that SMT7 and ALP expression are significantly correlated with MyoD expression ($p = 0.017$ and 0.035 , respectively). All of these data suggest that: MyoD is upregulated earlier, and to a greater extent, in FSHD myoblasts, and that its upregulation is correlated with turning on the myogenic program. Therefore the upregulation of p21 is likely to represent the logical continuation of the normal myogenic program in FSHD myoblasts. Because the behavior of FSHD myoblasts resembles that of control myoblasts undergoing replicative senescence, we wish to establish whether FSHD myoblasts have experienced true replicative senescence, or whether their loss of replicative capacity is based on other mechanisms disrupting the normal cell cycle. We have quantitated centromeric and telomeric DNA of myoblast samples from FSHD patients, muscle disease controls, and normal controls. The T/C ratio would be expected to be significantly less in FSHD myoblasts if muscle satellite cells had undergone a greater number of divisions prior to muscle biopsy. Thus far, our data do not support this. Funded by NIAMS 08R1AR48340A-01.

The Thoracoabdominal Syndrome (TAS): Narrowing the critical interval region and searching for the TAS gene.

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TAS presents a closure defect confined to the ventral midline. The main clinical manifestations of this syndrome are closure defect of the midline abdominal wall (ventral hernia) seen in all affected individuals, as well as a defect in the antero-lateral diaphragm manifested almost exclusively in affected males. TAS has been described in only one Jewish-Lybian family by our group and the mode of inheritance suggested from the familial segregation of anomalies is X-linked dominant. Linkage analysis study of 27 individuals of the Jewish-Lybian TAS kindred finely localized the TAS genes to Xq27, with no crossing over events with DXS1232 and DXS984, which are located approximately 350Kb apart. Lod scores of 5.49 and 6.36 were obtained for DXS1232 and DXS984, respectively. The TAS interval was thus defined between markers F9 on the centromeric side and DXS1205 at the telomeric side (about 1.6Mb), each showing a crossing over event in a different individual. The sequencing of the interval is almost completed with only two possible gaps. In order to narrow down the critical interval we have developed 15 additional polymorphic markers. On the centromeric side a new polymorphic marker narrowed the interval by 355Kb. Another polymorphic marker 328Kb telomeric to DXS984 showed no crossing over events. Further telomeric to this site there is a segmental duplication that prevents from developing additional polymorphic markers. Thus the telomeric border remains DXS1205. Two candidate genes in the critical interval: CDR1 and SPANXB1 were screened for mutations in an affected male by PCR amplification of the coding exons and their borders. No mutations were identified. Half of the coding region from the N-terminal side, including the HMG box of the SOX3 gene showed no mutations. 24 additional ESTs were identified in the critical interval that are now under study.

Development of a high-sensitive and low-background human muscle cDNA microarray to study muscular dystrophy. *T. Tsukahara*¹, *S. Noguchi*^{1,2}, *M. Fujita*^{1,2}, *R. Kurokawa*^{1,2}, *A. Tsujimoto*³, *I. Nishino*^{1,2}. 1) Dept Neuromuscular Research, Natl Inst Neuroscience, NCNP, Tokyo, Japan; 2) CREST, JST, Japan; 3) DNA Chip Research Inc., Yokohama, Japan.

The comprehensive gene expression analysis in disease muscles should be a powerful tool to understand the molecular pathomechanism of the muscle disease. However, there are two major problems in hybridization-based gene expression analysis: 1) Possibility of cross-hybridization of probes with homologous genes may not be negligible. 2) Huge amount of samples are necessary and thus a single muscle biopsy specimen is not enough for the analysis. To overcome these difficulties, we newly developed a human muscle cDNA microarray with high sensitivity and reliability. To make probes, we collected all sequence data annotated to be expressed in skeletal muscle from public databases. Sequences homologous with rRNA, mtDNA, and repetitive sequences were excluded from candidates. PCR primers were designed so as to specifically amplify the fragments of 450-550bp in length in the vicinity of 3 ends of each singleton. Each fragment in the database was amplified from muscle cDNA pools and confirmed by sequencing. We obtained 5,310 muscle-expressed genes including all known causative gene for muscular dystrophies and their associated genes. Amplified clones were purified and spotted onto a PLL-coated slide glass. We used a tyramide signal amplification system to enhance the signal. In triplicate experiments using human skeletal muscle total RNA as a target, our microarray demonstrated good reproducibility ($R=0.94-0.98$) in the range of the 100-100,000 relative fluorescence intensity. The fluorescence intensity of each target spot was linearly increased in the range of 1-4 microgram of total RNA, indicating that one microgram of total RNA was enough for the analysis. With our new cDNA microarray, we successfully obtained gene expression profile from less than mg of a single frozen muscle biopsy specimen. Our data were basically compatible with the previously published data, indicating that our cDNA microarray is a powerful tool to analyze molecular pathology of the disease in each patient.

Molecular analysis of *PTPN11* gene encoding protein tyrosine phosphatase SHP-2 and phenotype-genotype correlation in Japanese patients with Noonan syndrome. *Y. Aoki*¹, *T. Niihori*¹, *X. Yang*¹, *K. Kurosawa*², *H. Ohashi*³, *T. Kondo*⁴, *E. Ishii*⁵, *Y. Suzuki*¹, *S. Kure*¹, *Y. Matsubara*¹. 1) Department of Medical Genetics, Tohoku University School of Medicine, Sendai, Japan; 2) Division of Medical Genetics, Kanagawa Children's Medical Center, Yokohama, Japan; 3) Division of Medical Genetics, Saitama Children's Medical Center, Saitama, Japan; 4) Department of Pediatrics, Nagasaki University of Medicine, Nagasaki, Japan; 5) Department of Pediatrics, Saga Medical School, Saga, Japan.

Noonan syndrome (NS) is characterized by short stature, characteristic facial appearance including hyperterolism and ptosis, various congenital heart defects, broad or webbed neck, and mild mental retardation. Recently, missense mutations in *PTPN11*, the gene encoding protein tyrosine phosphatase SHP-2 (src homology region-2 domain phosphatase-2), were detected in approximately 50% of familial or sporadic patients with NS. Mutations detected in these patients were clustered in amino-terminal src-homology 2 (N-SH2) and protein tyrosine phosphatase (PTP) domains, implying that they were gain-of-function mutations. To identify mutations in NS patients from different ethnic background, we analyzed 20 Japanese patients with familial or sporadic NS. Fifteen coding exons with adjoining intronic sequences were amplified by PCR and sequenced. One novel mutation (Ser502Thr) and 7 previously reported mutations were found in 9 of 20 unrelated patients analyzed. Phenotype-genotype analysis showed that pulmonary stenosis was more prevalent among the patients with *PTPN11* mutations than those without mutations (7/9 vs. 4/11). The patient with the Ser502Thr mutation had an episode of transient abnormal myelopoiesis at 3 months of age and was associated with neuroblastoma at 6 months, suggesting the extending characteristics of the mutation. These results confirm the previous reports that missense mutations in *PTPN11* gene are responsible for NS in the different ethnic background.

Growth hormone gene deletions resulting from unequal homologous recombination enhanced by the presence of chi-like sequences. *C.T. Moseley¹, M.D. Orenstein², J.A. Phillips III¹*. 1) Department Pediatrics, Vanderbilt University School of Medicine, Nashville, TN; 2) Martin Luther King Magnet High School for Engineering and Health Sciences on the Pearl Campus, Nashville, TN.

Familial growth hormone deficiency type 1A is an autosomal recessive disease caused by deletion of both growth hormone-1 (GH1) alleles. To determine the location of crossover sites that resulted from unequal homologous recombination within the human GH gene cluster causing GH1 gene deletions, we mapped the breakpoints in six unrelated subjects who were homozygous for 6.7-kilobase (kb) deletions that encompassed GH1 but had different RFLP haplotypes. To do this, we designed primers to amplify a 1.2-kb deletion junction fragment for each subject. Restriction map and sequence analyses localized the breakpoints for all six subjects within two blocks of highly homologous DNA sequences that lie 5' and 3' to the GH1 gene. In five of these six cases, the unequal homologous recombination event occurred between two 79-bp perfect repeats that were 3329-bp 5' and 3284-bp 3' to the GH1 capsite. In the sixth subject, the recombination occurred between two 64-bp perfect repeats that were 4113-bp 5' and 2498-bp 3' to the GH1 capsite. Interestingly, none of the repeats contained Alu sequences. Further, the 79-bp and 64-bp repeats were extremely short compared to other repeat sequences also found within these two blocks of homologous DNA. A search for recombination-prone motifs revealed chi-like sequences in the 64-bp (5' - TCTGGTAA - 3') and the 79-bp (5' - CATGGTGG - 3') breakpoint regions. Our findings suggest that the chi-like sequences may be important in defining sites for homologous recombination that cause GH1 gene deletions.

CAG/CTG repeat expansions at the HDL2 locus are a common cause of Huntington disease in black South

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Huntington disease (HD) is an autosomal dominant neurodegenerative disorder, typically presenting with choreiform movements and subcortical dementia. A CAG repeat expansion in the IT15 gene on chromosome 4p is responsible for most cases of HD. Recently a CAG/CTG repeat expansion in the junctophilin-3 (JPH3) gene on chromosome 16q23 has been implicated in a subset of clinically diagnosed HD cases that did not have an expansion in the HD gene. The disorder caused by this mutation has been designated Huntington disease type 2 (HDL2). All HDL2 positive patients to date have been of African or African-American ancestry, with the exception of one Moroccan and one Mexican patient. In a sample of white South African individuals tested for HD, 75% (57/76) of individuals had expansions. Only 41% (14/34) of black South African patients, ascertained through the same referral system, were positive for HD expansions. Individuals who tested negative for the HD repeat expansion were tested for the HDL2 expansion. In the white South African group, to date 0% (0/6) of individuals tested positive. However, 35% (7/20) of black South African individuals tested positive, with repeat lengths between 40 and 49 (unaffected 8-28), confirming a diagnosis of HDL2. Clinical features in the 7 positive HDL2 cases are indistinguishable from HD. Age at presentation ranged from 47 to 62 years. At least 3 of these individuals have a positive family history of a Huntington-like disease. HDL2 mutations account for 21% of Huntington disease in the black South African individuals tested, suggesting that this locus contributes significantly to the HD phenotype in this population. It should thus be incorporated into routine HD diagnostic testing. Further, these findings suggest that one of the origins of the HDL2 mutation may have been in Africa.

Genetic heterogeneity of Huntington's Disease: screening of mutations in the DRPLA and TBP genes, HDL-1 and

-2 loci. *G. Stevanin¹, H. Fujigasaki¹, A.S. Lebre¹, C. Jeannequin¹, A. Camuzat¹, R.L. Margolis², C. Dode³, A. Brice^{1,4}, A. Durr^{1,4}.* 1) INSERM U289 and Institut Federatif des Neurosciences, Groupe Hospitalier Pitie-Salpetriere, Paris, France; 2) John Hopkins University School of Medicine, Baltimore, MD, USA; 3) Laboratoire de Biochimie et Genetique Moleculaire, Groupe Hospitalier Cochin-Port Royal, Paris, France; 4) Federation de Neurologie et Departement de Genetique, Cytogenetique et Embryologie, Groupe Hospitalier Pitie-Salpetriere, Paris, France.

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder resulting mainly from the loss of neurons in the striatum. Patients suffer from progressive and unremitting chorea, rigidity and cognitive impairment. A CAG repeat expansion in the IT15 gene on chromosome 4 is responsible for the vast majority of cases with HD phenotypes. This mutation accounts for 88% of HD cases in our series. We identified 88 HD patients without expansions in the IT15 gene and analyzed them for mutations previously reported in Huntingtons disease-like patients : i) a 192 nucleotide insertion in the PRPN gene encoding the prion protein (HDL1 locus) ii) a CTG/CAG repeat expansion in the gene encoding Junctophilin-3 (HDL2 locus) iii) CAG expansions in the DRPLA and TBP genes, reported in patients presenting with cerebellar ataxia and chorea and/or dementia and behavioral disturbances. CAG expansions in the DRPLA gene and insertions in the PRPN gene were not found in our series. On the contrary, expanded repeats at the HDL2 locus and in the TBP gene accounted for 2 and 4 patients with typical HD, respectively. HDL2 patients came from Morocco and French West Indies and carried 49 and 43 repeats. Both presented with chorea and dementia associated with cerebellar symptoms in one. Expansions of 43 to 49 repeats in the TBP gene were detected in 4 European HD-like patients. Dementia was the common feature of these patients and was associated with: ataxia and myoclonia (n=1), chorea (n=1), or with ataxia and chorea (n=2). In conclusion, repeat expansions at the HDL2 and TBP loci account for less than 1% each, in our series of HD-like patients. Further genetic heterogeneity is then expected.

Repeat analysis of the ZNF9 gene in DMPK expansion negative DM patients. *H. Scheffer¹, A. Tieleman², G. Padberg², M. de Visser³, M. Leferink¹, F. Hol¹.* 1) Human Genetics, Univ Medical Ctr Nijmegen, Nijmegen, Netherlands; 2) Neurology, Univ Medical Ctr Nijmegen, Nijmegen, Netherlands; 3) Neurology, Academic medical Ctr, Amsterdam, Netherlands.

Myotonic dystrophy (DM) is a multisystem disorder and the most common form of muscular dystrophy in adults. Patients exhibit myotonia, proximal and distal limb weakness, frontal balding, cataracts, and cardiac arrhythmias. One form of the disorder (DM1) is caused by an expanded CTG repeat in the 3 untranslated region of the DMPK gene. Recently, a second gene causing similar clinical symptoms was identified. DM2 is caused by a CCTG expansion located in intron 1 of the ZNF9 gene. Expanded allele sizes range from 75 to approximately 11,000 CCTG repeats, with a mean of approximately 5,000 repeats. We have analyzed the ZNF9 CCTG repeat in 12 unrelated DM patients presenting with the classical DM symptoms in whom a CTG DMPK expansion had been excluded. In 4 cases no expansion was identified, whereas in 8 cases a CCTG expansion was present. These results show that a substantial proportion of DM patients without DMPK CTG expansion is due to a ZNF9 CCTG expansion. Patients without DMPK or ZNF9 expansions may be caused by pathogenic alleles of a third locus. Alternatively, a different disease clinically resembling DM may in fact affect the patients in whom negative test results were obtained. The technical approach included fragment length analysis of amplified CCTG ZNF9 repeats in order to exclude DM2 by the presence of two normal length alleles. Subsequently, Southern analysis using part of the ZNF9 intron 1 as a probe was used. Since expanded alleles can be extremely large, and could be easily missed, it is recommended to compare the hybridization intensity of the normal allele with a reference probe. It can be noted that no somatic mosaics having two normal length as well as expanded repeat size ZNF9 fragments have been observed.

A new recessive syndrome of Cerebellar Ataxia with Saccadic Intrusions maps to 1p36. *M. Burmeister¹, S. Li¹, R.J. Leigh², I.N. Beshpalova¹, J. Weber³, B. Swartz².* 1) Mental Hlth Res Inst, Dept. of Psychiatry and Human Genetics, Univ Michigan, Ann Arbor; 2) Dept. of Neurology, Case Western Reserve University, Cleveland, OH; 3) Marshfield Medical Research Foundation, Marshfield, WI.

A family of Slovenian descent presented with at least 5 of 14 sibs affected by progressive ataxia, saccadic intrusions on fixation, axonal sensory neuropathy, and myoclonic jerks without EEG abnormalities. Although difficulties in reading and concentrating were reported, cognition appears unaffected. Disease onset was insidious, but early signs are remembered in their 20s. SCA1-3, 6-8, Friedreichs ataxia, EPM1 and MJD have been excluded by commercial mutation tests. Linkage to known recessive ataxias on 19p13.3 (Cayman Ataxia) and 21q (UL-PME) was excluded. A Genome Scan with 374 short tandem repeat polymorphisms was performed at Marshfield on the mother, 5 affected, and 4 unaffected offspring. For several markers linkage could not be excluded. On 1p36, additional markers (ABIs 5 cM genome scan panel, performed at U of M Sequencing Core) were tested on these plus one additional unaffected individual. Several markers were completely informative and nonrecombinant and showed linkage with a maximal LOD score of 3.03 at 0 cM. The nonrecombinant region is flanked by recombinant markers D1S468 and D1S507 and spans about 30 cM or about 13 Megabases. Candidate genes within the nonrecombinant interval are KCNAB2, a K-Channel which when knocked out in mice leads to decreased life expectancy, occasional seizures and myoclonic jerks after cold water stress, and Kinesin KIF1B, dominant mutations of which have been shown to cause the sensory neuropathy syndrome Charcot Marie-Tooth Disease type 2A. Since all 4 grandparents came from the same valley in a remote area of Slovenia, homozygosity mapping is likely to allow further fine mapping, to be followed by candidate gene testing.

Genetic mapping and identification of a new autosomal nonsyndromic recessive deafness locus *DFNB37*. Z.M. Ahmed^{1,2}, R.J. Morell¹, S. Riazuddin¹, M.M. Ahmad¹, S.A. Mohiddin³, R.C. Caruso⁴, S.N. Khan², L. Fananapazir³, T. Husnain², A.J. Griffith^{1,5}, S. Riazuddin², T.B. Friedman¹, E.R. Wilcox¹. 1) Section on Human Genetics, LMG, NIDCD,NIH, Rockville, MD; 2) National Center of Excellence in Molecular Biology, Punjab University, Lahore, Pakistan; 3) Clinical Cardiology Section, NHLBI, NIH, Bethesda, MD; 4) Section on Ophthalmic Molecular Genetics, NEI, NIH, Bethesda, MD; 5) Section on Gene Structure and Function, LMG, NIDCD, NIH, Rockville, MD.

Autosomal recessive profound nonsyndromic deafness in a large family from Pakistan (PKDF10) was found during a genome-wide scan to co-segregate with markers on chromosome 6q, defining *DFNB37*. A fully linked marker supported a lod score of 6.53 at $q=0$. Subsequently, linkage to *DFNB37* was found in two additional families segregating recessive nonsyndromic deafness from Pakistan (PKDF71,PKSR14). All three families are unrelated and carry different haplotypes for the markers in the linkage region. In addition to pure-tone audiometry, participating family members underwent medical history interviews, balance tests, and, in a subset of hearing impaired subjects, funduscopy, ENG and ERG exams were performed. Affected individuals from PKDF10 had a delay in developmental milestones and an abnormal ENG, confirming a malfunctioning vestibular organ. Upon sequencing a candidate gene from the *DFNB37* linkage region, a single base pair insertion in the open reading frame, predicted to cause a frameshift and premature termination, was homozygous in affected individuals of family PKDF10. Affected individuals of family PKDF71 have a homozygous nonsense mutation in the second to the last exon of the same gene. The deaf individual of family PKSR14 has a homozygous missense mutation. This mutation was not found among 351 normal representative DNA samples from Pakistan or from the Human Diversity panel (C.C.R.). The two other mutations were not found by sequence analysis among DNA from 100 normal control samples. More than 200 families segregating recessive deafness from Pakistan and India are currently being screened for *DFNB37* to find the relative contribution of this gene to deafness in Southeast Asia.

Linkage and mutation analysis of a family with autosomal dominant late onset macular degeneration. R.

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PURPOSE: To map and clone the gene causing a unique late onset autosomal dominant macular degeneration in a large five-generation family with prominent atrophic and exudative features closely resembling those found in age-related macular degeneration (AMD) and Sorsby fundus dystrophy (SFD). **METHODS:** Phenotype was characterized by fundus photography, fluorescein angiography, visual field testing, ERG and visual function tests on selected individuals and by reviewing ophthalmologic data on others. Microsatellite markers were analysed using standard protocols. Linkage analysis was carried out using MLINK program. Mutation analysis of candidate genes was carried out by PCR amplification of exons followed by direct sequencing. **RESULTS:** All the affected individuals displayed characteristic late onset atrophic macular changes with or without superimposed choroidal neovascularization. Disease symptoms are first manifested in later half of 5th decade. We obtained blood samples from 17 affected members, 17 unaffected and 5 spouses. Linkage, haplotype and mutation analysis have excluded the *TIMP3*, *Bestrophin*, *ELOVL4*, *EFEMP1* genes associated with macular degenerations. Significant negative LOD scores were obtained with markers linked to Stargardt's macular degeneration, Age related macular degeneration, North Carolina macular degeneration loci. Analysis with additional markers has given significantly high positive LOD scores suggesting linkage. Additional markers linked to this region are being tested to confirm linkage. **CONCLUSIONS:** We identified a family with late onset macular degeneration phenotype that mimics AMD and SFD. We have excluded most of the previously known candidate gene loci by linkage and mutation analysis.

A 288 bp exonic insertion and novel missense mutations in PAX9 in families with autosomal dominant

hypodontia. *P. Das*¹, *M. Hai*², *C. Elcock*³, *S.M. Leal*³, *D.T. Brown*⁴, *A.H. Brook*³, *P.I. Patel*^{1,2}. 1) Dept Neurology, Baylor Col Medicine, Houston, TX; 2) Dept of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Dept of Oral Health and Development, School of Clinical Dentistry, Clarendon Crescent, Sheffield S10 2TA, UK; 4) 5001 Highway 190, Suite A6, Covington, LA 70433.

We previously reported a frameshift mutation in PAX9 as an underlying cause for familial autosomal dominant oligodontia involving posterior teeth (Nature Genet 24: 18-19, 2000). More recently, we reported a submicroscopic deletion (~56 Kb) encompassing PAX9 and SLC25A21 in a small nuclear family where a father and his daughter were affected with severe hypodontia involving both primary and permanent molars (Hum Genet, 110: 371-376, 2002). Since PAX9 was the only odontogenic gene within this deletion, we concluded that haploinsufficiency for PAX9 is the underlying cause for hypodontia. Here we report the molecular analysis of three additional families with hypodontia. In two of these families (DEN 3 and DEN9), PAX9 was associated with the disease phenotype by linkage analysis. DHPLC analysis followed by DNA sequencing in affected members of these two families revealed missense mutations, namely, a leucine substituted by proline in family DEN3 and a lysine substituted by glutamic acid in DEN9, within the paired domain of PAX9. A 288 bp insertion within exon 2 of PAX9 was detected in two identical twins affected with hypodontia in the third family, resulting in a putative frameshift mutation and a premature stop codon. These studies extend the spectrum of mutations in PAX9 associated with hypodontia, and include the categories of heretofore-undescribed missense mutations and a large exonic insertion.

EVIDENCE FOR A GENETIC HETEROGENEITY OF MEGALOBLASTIC ANAEMIA 1 IN TUNISIAN PATIENTS. *C. Bouchlaka*¹, *C. Maktouf*¹, *T. Sfar*², *A. Ayadi*², *A. M'kaour*³, *M. Sioud*³, *N. Gueddich*⁵, *Z. Belhadjali*⁴, *K. Dellagi*¹, *S. Abdelhak*¹. 1) Immunology, Inst Pasteur, Tunis, Tunisia; 2) C.H.U, Tahar Sfar, Mahdia, Tunisia; 3) hôpital d'enfants, Tunis, Tunisia; 4) C.H.U, Aziza othmana, Tunis, Tunisia; 5) C.H.U, Monastir, Tunisia.

Imerslund-Grasbeck syndrome or megaloblastic anaemia 1 (MGA1) is a rare autosomal recessive disorder characterised by a selective intestinal cobalamine malabsorption. MGA1 occurs worldwide, but its prevalence is high in Norway, Finland and several Middle Eastern countries. In Tunisia, more than 20 patients have been diagnosed. The locus MGA1, has been previously mapped by linkage analysis in Finish and Norwegian families to a 6 cM region on chromosome 10p12.1. The gene "CUBN" encoding the Intrinsic factor (IF)-vit B12 receptor, also called cubiline, was recently identified by positional cloning. Two specific mutations have been described in 17 finish MGA1 patients. P1297L was the most prevalent mutation. Involvement of "CUBN" gene in MGA1 was investigated by linkage analysis in Tunisian families. Eight patients belonging to six consanguineous families have been included in this study. Segregation analysis was performed using four microsatellite markers (D10S1476- D10S504- CUBN - D10S1714) overlapping the "CUBN" region. Haplotype analysis and homozygosity mapping have shown that in four families, the patients were homozygous by descent for all the markers, suggesting a likely linkage to MGA1 locus. These patients have been screened for the P1297L mutation by direct sequencing. No one of our patients bared this prevalent finish mutation. One patient belonging to a consanguineous family was heterozygous for all the markers including the intragenic marker (CUBN) but his haplotype differed from his unaffected brother. This patient may be a compound heterozygote or probably did not cosegregate with MGA1 locus. Another patient born to a consanguineous family was also heterozygous for all the markers and share the same haplotype as his unaffected brother, excluding linkage to MGA1 locus. These results suggest a genetic heterogeneity in MGA1, this has been already suggested by genetic studies of a canine model.

What Defines a Critical Region for Gene Finding? *W.S. Davidson, L.C. Wilkins.* Molecular Biology & Biochemistry, 8888 University Drive, Simon Fraser University, Burnaby, BC, V5A 1S6 Canada.

Linkage analysis has traditionally been the method used to define a locus associated with a genetic disease. The location of the gene is refined on the basis of advantageous recombination events that permit the identification of regions that are shared by affected individuals and excluded from unaffected individuals in the same kindred. Even within a large extended family, recombination events are infrequent and thus the critical region is usually too large for immediate positional cloning of the gene. In order to reduce the critical region to a size that is amenable for gene identification and the search for the mutation(s) causing the disease, one normally has to rely on a combination of cross-overs in different families. We encountered problems with this standard approach during the search for the gene associated with autosomal recessive kidney disease (ARPKD). Our approach involved analysing a known consanguineous Newfoundland family in which a recombination event reduced the previously described critical region from one end. Using a combination of microsatellite markers and single nucleotide polymorphisms, it was possible to reduce the region of homozygosity in affected individuals within the previously defined critical region to 230 kbp. Several gene prediction approaches identified 13 potential exons. Although sequence variants were observed in these regions of the DNA from family members, no mutation associated with the disease was found. The gene for ARPKD (PKHD1) has recently been described and 10 of our predicted exons corresponded to exons 52-61 of PKHD1. We have subsequently identified the homozygous mutation in this kindred. The mutation resides in exon 62 of PKHD1 which is outside the critical region defined using the standard methodology described above. Our experience with this problem should alert the gene finding community that, when using multiple families to define critical regions, one must consider the possibility that the mutation in a particular family can reside outside what may be considered the standard critical region. (Funding for this work was provided by the Canadian Institutes for Health Research).

A New Gene for Autosomal Dominant Cerulean Cataract Type-3. *V. Kumar¹, D. Singh², K. Sperling³, J.R. Singh¹.*
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Purpose: Gene mapping by linkage analysis in an autosomal dominant congenital cataract (ADCC) family. **Methods:** Gene mapping studies were carried out in an autosomal dominant congenital cataract family of Indian origin, having 12 members in 3-generations, as affected with cerulean cataract. More than 100 fluorescently labelled microsatellite markers, selected from known candidate gene regions, were used for the analysis. Genotyping was done with GENOTYPER 2.5.1 software on ABI-377 sequencer. 2-Point and multipoint linkage analysis were carried out using the MLINK and LINKMAP components of the linkage program package ver. 5.1 respectively. **Results:** On the basis of obtained 2-point and multipoint lod score values, we initially excluded the known loci for cerulean cataract type-1 and type-2 at 17q24 and 22q11.2-12.1 respectively. Further the other known candidate gene regions on chromosomes 1, 2, 3, 9, 10, 11, 12, 13, 14, 15, 17, 19, and 21 were also excluded, as the obtained 2-point lod score values were less than 2 at $\alpha=0.001$, except for the markers on chromosome 16. A maximum positive LOD score of 3.9 at $\alpha=0.000$ indicative of linkage, was obtained with 3 markers on chromosome 16. Multipoint analysis carried out with these analysed markers also supported the linkage, with a maximum LOD score of 3.612 between markers D16S518 and D16S511. Multipoint and haplotype analysis narrowed the cataract locus to a 16.6 cM region between markers D16S3097 and D16S511 that corresponds to the 16q22 region on chromosome 16. **Conclusion:** This is the first report of mapping a gene for cerulean type cataract, at 16q22. Since the gene for cerulean cataract type-1 and type-2 have already been mapped at 17q24 and at 22q11.2-12.1 respectively, the present study further supports the genetic heterogeneity for this type of cataract. On the basis of localization of Marner type and posterior polar cataract to the same region i.e. 16q22, the possibility of allelic heterogeneity in congenital cataract cannot be excluded.

Evidence for further genetic heterogeneity in posterior polymorphous dystrophy. *E. Heon*¹, *C.E. Willoughby*^{1, 2}, *G.D. Billingsley*¹, *H. Herbert*², *S.B. Kaye*². 1) Ophthalmology, Vision Science Research Program, Toronto Western Hospital Research Institute, Univ Toronto, Toronto, ON, Canada; 2) Royal Liverpool University Hospital, Liverpool, UK.

Posterior polymorphous dystrophy (PPD; OMIM#122000) is a progressive disorder of the corneal endothelium which is normally dominantly inherited. The corneal endothelial cells develop epithelial characteristics in PPD and provide hallmark pathological findings. We identified mutations in the VSX1 homeobox gene have been identified in PPD and keratoconus, and mutations COL82A have been described in PPD and Fuchs endothelial dystrophy. The aim of this study is to document the spectrum of the ocular phenotype and genetically characterize a three generation pedigree with PPD. Eleven family members (5 affected and 6 unaffected) had a full ocular assessment including corneal topography and pachymetry. The proband underwent penetrating keratoplasty (corneal transplantation) allowing histological confirmation of the diagnosis. The corneal material was examined by light microscopy and demonstrated positive immunohistochemical staining for cytokeratins. Microsatellite markers for the VSX1 and COL82A loci were selected and genotyping performed on a Pharmacia automated sequencer. Two-point linkage analysis used the MLINK program of the LINKAGE package v5.2. Linkage to VSX1 and COL82A was excluded in this family with histologically confirmed PPD. The clinical and genetic analysis of this family further outlines the genetic heterogeneity of PPD.

Evidence consistent with linkage to 15q of a non-chromosome 4 linked FSHD family. *B.L. Randolph-Anderson, J.M. Stajich, F.L. Graham, M.A. Pericak-Vance, M.C. Speer, J.R. Gilbert.* Ctr Human Genetics, Box 3445, Duke Univ Med Ctr, Durham, NC.

Facioscapulohumeral muscular dystrophy (FSHD; MIM 158900) is a primary disease of muscle with early symptoms including facial or shoulder girdle weakness and subsequent involvement of pelvic girdle and extremity muscles as the disease progresses. The majority of FSHD families are linked to the 4q35-qter region (FSHD1A), although genetic heterogeneity (FSHD1B) has been established within this diagnostic classification. We have identified two FSHD families in which non-linkage to chromosome 4 has been confirmed (DUK1361 and DUK2531). Genomic screening has identified a region on chromosome 15 consistent with linkage. Several markers in an 18cM region on chromosome 15 provided positive lod score values. Multipoint analysis gave supporting evidence for the results revealed in the two point data analysis, with a peak lod score of 3.20 in these families. While haplotype analysis suggests that the most likely interval spans D15S1004 and D15S536, one individual in family 1361 tentatively classified as affected (individual 9019) in this pedigree excludes the region in its entirety. Initial analysis of the D4Z4 repeat indicates that this affected individual has a repeat length of 30 kb; however, haplotype analysis of the pedigree conclusively eliminates the 4q region in this family. These data raise the possibility that this affected individual may be a phenocopy. A search for candidate genes in the region identified POLG, which encodes the gamma subunit of mitochondrial DNA polymerase. Mutations in this gene are responsible for the myopathic disease progressive external ophthalmoplegia. We have investigated POLG for evidence of mutation by PCR sequence analysis of the 22 coding exons and the promoter region in two affected individuals. To date, these investigations suggest that POLG is not the gene responsible for FSHD1B in this family. Further investigations of genes in this region are underway.

A Novel Homozygous Fusion Allele in a Patient with Type 2 Gaucher Disease. *N. Tayebi, B. Stubblefield, E. Sidransky.* Section on Molecular Neurogenetics, NIMH, NIH.

The deficiency of the lysosomal enzyme glucocerebrosidase results in Gaucher disease, an autosomal recessive inherited disorder. Based upon the presence and the progression of neurological symptoms, Gaucher disease is divided into type 1, non-neurologic, type 2, acute neurologic and type 3, subacute neurologic forms. The human glucocerebrosidase gene (GBA) on 1q21 has a pseudogene sharing 96% sequence homology located 16 kb downstream of the functional gene. We have studied patients with type 2 Gaucher disease in detail, utilizing molecular techniques including PCR, long-template PCR, direct sequencing, southern blot and polymorphic site analyses. Among 37 patients with type 2 Gaucher disease, 18 recombinant alleles (24%) were identified in 14 patients. These alleles arise from a recombination between GBA and its pseudogene after unequal pairing between the 2 chromosomes. Among 4 patients homozygous for recombinant alleles, 3 patients carried a short stretch of pseudogene sequence, ranging from 824-1193 bp, resulting from a gene conversion event. Based on sequencing and southern blot analyses, the fourth, a 19 week fetus, showed homozygosity for a fusion allele. The site of crossover in the sequence for both alleles occurred within the last 186 bp of exon 9 or at the beginning of intron 9. A true homozygous fusion allele has not previously been demonstrated. However, since parental DNA samples were not available for this case, the possibility of a deletion of one allele remains. DNA rearrangement events between the gene and pseudogene in the GBA locus appear to be lethal events when they occur on both alleles. Genetic studies of neuronopathic patients with Gaucher disease should be performed in great detail, to more accurately describe the recombinant alleles and to better resolve the relationship between genotype and phenotype in this disorder.

The mitochondrial leader sequence is present in all OPA1 mRNAs expressed in human brain. *M. Fiket, C. Alexander.* Neuroscience, Max-Delbrueck-Center, Berlin, Germany.

Autosomal dominant optic atrophy (ADOA) is the most prevalent hereditary optic neuropathy resulting in progressive loss of visual acuity, centrocoecal scotoma, and bilateral temporal atrophy of the optic nerve with an onset within the first two decades of life. This disease is caused by mutations in the OPA1 gene, which was identified by a positional cloning approach. The gene is located on chromosome 3q28 and is split into 31 exons. Examination of the N-terminal leader sequence of the deduced protein sequence revealed the typical features of a protein imported into the matrix space of mitochondria, which has been experimentally proven by reporter gene assays. Purpose: Preliminary northern blot analysis revealed the presence of OPA1 transcripts in all tissues examined but at varying abundances and sizes (Alexander et al. 2000). Minor differences in length (3-270 bp) can be accounted for by a splicing hot spot involving exons 4, 4b, 5, 5b (Delettre et al., 2001), but an explanation for the major size differences observed in the range of kilo bases was lacking. We sought to examine the sequence content of all OPA1-mRNA species observed by northern blotting in the brain. Especially, we were aiming at elucidating whether all OPA1 mRNAs expressed in the human CNS contained the sequence part coding for the mitochondrial import motif. Methods: PolyA+mRNA samples of more than 10 different human brain areas were examined by northern blot hybridisation. Radioactively labelled probes corresponding to different protein domains of the human OPA1-cDNA (AB011139) were used in the hybridisations. Results: Our experiments revealed the same multiple-band pattern in all tissue samples examined. Four different mRNA species of a length of 4, 5, 6, and 7 kb were identified. The sequence encoding the mitochondrial import motif was present in all of these transcript variants. Conclusion: We can conclude that all four OPA1 transcript species harbour the mitochondrial leader sequence. Thus, all putative OPA1 protein isoforms in the human brain are imported into mitochondria.

A missense mutation (I314L) in the homeodomain of HOXD13 results in both gain- and loss of DNA binding activity. *S. Kan*¹, *D. Johnson*^{1,2}, *H. Giele*², *A.O.M. Wilkie*^{1,2}. 1) Weatherall Institute of Molecular Medicine, The John Radcliffe, Oxford, UK; 2) Plastic & Reconstructive Surgery, Radcliffe Infirmary, Oxford, UK.

The HOX genes encode a highly conserved family of transcription factors, several of which act in a sequential and concerted manner to play fundamental roles in limb development. To date, two HOX genes have been proven to be mutated in human limb disorders: *HOXD13* in synpolydactyly (SPD) and *HOXA13* in hand-foot-genital syndrome.

We screened for mutations of the *HOXD13* open reading frame in patients with limb malformations, and identified a novel heterozygous mutation (940A>C, I314L) in all affected members of two pedigrees. The hand phenotypes of the two families are variable, including brachydactyly of the 5th digit, partial duplication of the 4th digit, and shortening of the 3rd metacarpal. The I314L mutation locates in the α -helix III of the conserved homeodomain at position 47. This is one of the four amino acid residues shown to make specific protein-DNA contacts in a variety of homeodomain-DNA crystal structures. Biochemically, isoleucine and leucine share many similar characteristics; however, in a cross-species line up of 364 different homeodomain-containing genes, leucine was never observed at homeodomain position 47.

Previous work has identified a core 5'-TTAC/T-3' (b-strand) consensus binding motif for proteins encoded by the 3' HOX genes. We compared the binding activity of the I314L mutant HOXD13 with wild type using gel shift assays. The I314L mutant bound the TTAC motif with higher affinity, but TTAT with lower affinity, compared to wild type. These findings suggest that the I314L mutation may differentially enhance or diminish the binding of HOXD13 to different DNA sequences and thereby exert both gain- and loss-of-function effects through different pathways; this explains why the phenotype associated with this mutation differs from SPD. The finding that the Leu47 HOXD13 homeodomain retains DNA binding activity raises interesting questions about why it is not observed in nature.

Characterization of missense mutations in the forkhead transcription factor FOXC1, a gene mutated in Axenfeld-Rieger malformations. *R.A. Saleem, F.B. Berry, T.C. Murphy, M.A. Walter.* Medical Genetics/Ophthalmology, Univ Alberta, Edmonton, AB, Canada.

FOXC1 is a developmentally important transcription factor. Mutations in FOXC1 lead to aberrant development of the anterior segment of the eye and increase the risk of glaucoma. We have previously determined the consequences of 5 missense mutations on FOXC1. Recently, five new FOXC1 missense mutations underlying AR malformations have been identified (P79L, P79T, I91S, I91T, R127H). The effects of these FOXC1 mutations on the ability of the FOXC1 protein to bind and bend DNA and activate gene expression were investigated. Site-directed mutagenesis was used to introduce these missense mutations into the FOXC1 cDNA. The effect of each additional missense mutation on FOXC1-DNA interactions was tested by electrophoretic mobility shift assays (EMSAs) and DNA-bending assays, while the effect of each missense mutation on the transactivation ability of FOXC1 was tested using a dual luciferase reporter assay. Preliminary results indicate that while all the missense mutations tested demonstrate reduced transactivation of a luciferase reporter gene in comparison to wild type FOXC1, the levels of residual activity of the mutant forms of FOXC1 vary greatly. Additionally, EMSAs have revealed that FOXC1 carrying P79L, P79T, I91S, or I91T is still able to bind an *in vitro* derived FOXC1 binding site at near wt FOXC1 levels. Further analyses of the effects of these missense mutations on FOXC1 binding specificity and the nature of the transactivation disruption are underway. Combined with our previous work, these experiments show that missense mutations in FOXC1 are able to disrupt the stability of FOXC1 protein, the interaction of FOXC1 with DNA, and/or the transactivation potential of FOXC1. This study will provide an understanding of how disease-causing mutations perturb FOXC1 function and will define critical thresholds of FOXC1 function. These studies indicate that FOXC1 protein levels and activity are tightly controlled and will provide a basis for understanding the role of FOXC1 in normal development.

Analysis of candidate genes for Crohn's disease: association with IL-4R and STAT6 genes from the IBD1 and IBD2 region. *B. Franke*¹, *D.J. de Jong*², *A.H. Naber*², *J.J. Willemen*¹, *J.G. Heister*¹, *H.G. Brunner*¹, *F.A. Hol*¹. 1) Department of Human Genetics, University Medical Centre Nijmegen, Nijmegen, The Netherlands; 2) Department of Gastroenterology and Hepatology, University Medical Centre Nijmegen, Nijmegen, The Netherlands.

BACKGROUND: Genetic susceptibility plays an important role in the etiology of Crohn's disease. Linkage studies indicate that genes in the IBD1 locus on chromosome 16 and (to a lesser extent) in the IBD2 locus on chromosome 12 are involved. Within the IBD1 region, NOD2 was recently identified as the gene of interest. However, linkage to the broad IBD1 region appears not to be explained by NOD2 alone. In IBD2, no involved genes have been described, yet. **AIMS:** In this study we tried to assess the relation of putative candidate genes in IBD1 (other than NOD2) and IBD2 with Crohn's disease. **METHODS:** Polymorphisms in the genes coding for IL-4R, CD11b, NOD2 (all three in IBD1) and STAT6 (in IBD2) were tested for association with Crohn's disease, using Transmission Disequilibrium Tests in patient-parent triads. **RESULTS:** In IBD1 we observed significant association with the Q576R polymorphism in IL-4R and a trend towards significance for the C-insertion allele of NOD2. IL-4R results remained significant upon exclusion of families with this NOD2 allele. No association was observed with the CD11b gene in IBD1. In IBD2 we found significant association of Crohn with a polymorphism in the STAT6 gene. **CONCLUSION:** IL-4R in the IBD1 region and STAT6 in the IBD2 region are associated with Crohn's disease. Both genes are functionally related in T lymphocyte differentiation and may play a role in the etiology of Crohn's disease. Our results indicate that IL-4R and NOD2 constitute independent risk factors for Crohn's disease in IBD1.

Two familial autosomal-dominant hemochromatosis cases associated with mutations in the SLC11A3 gene. V.

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Hemochromatosis is a progressive iron overload disorder frequent among subjects of European descent. It is generally inherited in an autosomal recessive pattern and most cases are related to homozygosity for the C282Y mutation in the HFE1 gene. We report here two familial cases of non HFE1 iron overload ; clinical symptoms of affected individuals were similar to those observed in HFE1 hemochromatotic patients but they did not have mutations in HFE1. Taking into account their particular genetic, biological and histological features, respectively 1) autosomal dominant inheritance, 2) marked increase in serum ferritin but moderate transferrin saturation, 3) iron accumulation in hepatocytes but also in Kpffer cells, the best candidate gene seemed to be SLC11A3 (Ireg1) recently implicated in similar cases. SLC11A3 encodes ferroportin, a basolateral membrane protein involved in the export of iron from the enterocyte to the circulation. Results : in the first family the proband and his son shared the same heterozygous deletion in exon 5, val162del. In the second one the three affected subjects (the mother and her two daughters) presented with the same heterozygous substitution in exon 8 resulting in the replacement of glycine 490 with aspartate. Conclusion : The involvement of the SLC11A3 gene should be evoked in autosomal dominant iron overload with major increase of serum ferritin.

Novel anterior segment phenotypes resulting from forkhead gene alterations: evidence for cross-species

conservation of function. *O.J. Lehmann*¹, *S. Tuft*², *G. Brice*³, *R. Smith*⁴, *A. Blixt*⁵, *A. Child*³, *T. Jordan*¹, *R.A. Hitchings*², *P.T. Khaw*², *S. John*⁴, *P. Carlsson*⁵, *S.S. Bhattacharya*¹. 1) Dept Molecular Genetics, Inst Ophthalmology, London, England; 2) Moorfields Eye Hospital, London, England; 3) Department of Cardiological Sciences, St. George's Hospital Medical School, London; 4) The Jackson Laboratory, Bar Harbour, Maine, USA; 5) Department of Molecular Biology, Gothenburg University, Gothenburg, Sweden.

Mutations in murine and human orthologs usually result in similar phenotypes. However with mutations in two forkhead transcription factor genes (*FOXC1* and *FOXC2*), inter-species differences include corneal or anterior segment phenotypes, respectively. To determine whether such discrepancies provided an opportunity for identifying novel human/murine ocular phenotypes, pedigrees with early onset glaucoma phenotypes secondary to segmental chromosomal duplications/deletions encompassing *FOXC1* and 18 individuals with *FOXC2* mutations underwent detailed ocular phenotyping. Mice with mutations in either *Foxc1* or *Foxe3*, were assessed for features of the human phenotypes. A significant increase in central corneal thickness was present in affected individuals from the segmental duplication pedigrees compared with their unaffected relatives (mean 13%, maximum 35%, $P < 0.05$). Alterations in corneal thickness were present in mice heterozygous and homozygous for *Foxe3* mutations but neither in *Foxc1* heterozygotes nor the small human segmental deletion pedigree. Mutations in *FOXC2* resulted in ocular anterior segment anomalies. These were more severe and prevalent with mutations involving the forkhead domain. Normal corneal development is dependent upon the precise dose and levels of activity of certain forkhead transcription factors. The altered corneal thickness attributable to increased forkhead gene dosage is predicted to affect clinical management of certain glaucoma subtypes through increasing recorded intraocular pressure. Taken together with the *FOXC2* mutation ocular phenotype, whose genotype-phenotype correlation may represent a feature common to other forkhead genes, these results highlight the remarkable cross-species conservation of function amongst forkhead genes.

Expression of mutant *JAGGED1*, which encodes a Notch ligand, in a fetus with Alagille syndrome. *M. Meunier-Rotival¹, C. Driancourt¹, J. Boyer¹, N. Raynaud¹, C. Crosnier¹, J. Deschatrette¹, M. Gonzales², M. Hadchouel^{1,3}.* 1) Genetique et maladies du foie de l'enfant, INSERM U347, Kremlin-Bicetre,; 2) Unite de Foetopathologie, Hopital Saint-Antoine, Paris; 3) Service d'Hepatologie pediatrique, Hopital de Bicetre, France.

Heterozygous mutations of *JAGGED1*, encoding a ligand for Notch receptors, are responsible for Alagille syndrome (AGS), a developmental disorder mainly affecting liver, heart and vasculature, vertebra, kidney and eye. The phenotype is variable even among patients with the same mutation. The mutations are found in the part of the gene encoding the extracellular and transmembrane domains of the protein. The majority of the mutations lead to a premature termination codon, which could produce a truncated protein. We previously demonstrated that both mutant and wild-type *JAGGED1* alleles are transcribed in lymphoblastoid cell lines of AGS patients, carrying missense, nonsense, frameshift, and splice site mutations. By studying a fetus carrying a frameshift mutation in the *JAGGED1* gene, we show that this is also the case in vivo. Prenatal molecular diagnosis was performed on a mother with AGS, due to a 4-bp deletion in exon 9 of *JAGGED1*. The fetus carried the same mutation. The parents elected to terminate the pregnancy. The 24-week-old fetus exhibited pulmonary stenosis, cystic right kidney, and thymic hypoplasia. We measured, by real time RT-PCR, the expression of *JAGGED1* in the liver, the kidney, and a cell line established from the aorta. The level of expression was 20 times higher in the kidney than in the liver. Both the wild-type and mutant *JAGGED1* RNAs were present in the 2 tissues, albeit at different concentrations. Similarly both wild-type and mutant alleles were transcribed in the cell line from aorta. These results are in good agreement with the expression pattern of *JAGGED1* in normal human embryos and fetuses, showing a strong expression pattern of *JAGGED1* in the cardiovascular system and in kidney, but no label in the bile ducts. The presence of the mutant transcript, even in relatively small quantities, indicates that the mutant (soluble) protein may be synthesised and act via a dominant negative mechanism.

Novel Missense Mutation Suggests the Heart is More Sensitive to JAG1 Dosage than the Liver. *J.J.D. Morrissette, F.M. Lu, R.P. Colliton, N.B. Spinner.* Division of Human Genetics, Children's Hospital of Phila., Philadelphia, PA.

Alagille syndrome (AGS) is caused by haploinsufficiency for JAG1, a ligand that functions in the Notch signaling pathway, which is involved in cell fate determination. AGS, is a variably expressed, dominant disorder affecting the liver, heart, eye, skeleton and face. The molecular basis for the variable expressivity is unknown, but no genotype phenotype correlation has been demonstrated. JAG1 mutations in AGS include whole gene deletions, protein truncating, splicing and missense mutations, suggesting that haploinsufficiency is the mechanism of disease causation. A few missense mutations have been studied, and were found to result in failure of the protein to be transported to the cell surface, consistent with a mechanism of haploinsufficiency. A unique JAG1 missense mutation (G274D) was previously identified in 13 individuals from an extended family who presented with cardiac defects in the absence of liver dysfunction (Eldadah et al., 2001). We studied the expression, localization and activity of this mutation to explain the organ specific phenotype in affected individuals. The G274D mutation was found to be conditional ("leaky") in that it produces two populations of proteins, normal and abnormal. The mutant population is abnormally glycosylated and is retained intracellularly, rather than being transported to the cell surface, similar to previously studied JAG1 missense mutations. The G274D-JAG1 protein is temperature sensitive, with more abnormal (and non-functional) molecules produced at higher temperatures. Carriers of this mutation therefore have more than 50%, but less than 100% of the normal concentration of JAG1 molecules on the cell surface. The cardiac specific phenotype associated with this mutation leads us to hypothesize that the developing heart has increased sensitivity to changes in JAG1 dosage, compared to the developing liver.

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FOXL2 gene deletions in patients with Blepharophimosis Ptosis Epicanthus Inversus Syndrome (BPES). V.S. Yellore, N. Udar, M. Chalukya, S. Yelchits, R. Silva-Garcia, K. Small. Dept Ophthalmology, Univ California, Los Angeles, Los Angeles, CA.

Blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) is an autosomal dominant disorder of the eyelids that occurs in either of two types. Type I BPES, but not type II, is associated with premature ovarian failure. The causative gene FOXL2, is a forkhead transcription factor and is located at chromosome 3q23. We sequenced the entire FOXL2 gene in 40 patients from independent families and found base changes leading to mutations in only 15 of these individuals. Deletion in the 3q23 region have been reported to cause BPES. Large deletions can be identified by FISH analysis but small deletions are more difficult to identify. We used microsatellite analysis to identify deletions in the remaining patients. Using genomic sequence we generated 6 new microsatellite markers flanking the FOXL2 gene extending 0.5 Mb on either side of the gene. We genotyped the 15 probands and their families for these markers. Using haplotype analysis we identified 3 patients to be hemizygous for these markers thereby establishing deletion of this region. The deletion mutations accounts for 7.5 of our patients population. This work contributes further evidence of haploinsufficiency leading to the syndrome.

Human Genome Variation: The Problems and Challenges Ahead. *R.G.H. Cotton*^{1, 2}, *O. Horaitis*¹ and & members of the *Human Genome Variation Society*. 1) Genomic Disorders Research Centre, Melbourne VIC 3065, Australia; 2) The University of Melbourne, Dept. of Medicine, Melbourne VIC 3010, Australia.

Variations in human genes are assuming an increasing importance in all areas of health care. New methods for the detection of mutations and the completion of the human genome sequencing project have seen an exponential rise in variation information that must be collected, quality controlled, documented and stored safely to ensure future availability to health care professionals, researchers and others. This task will be a major challenge for the future of biological and medical science as there may be anywhere from one to greater than 1000 mutations in any given gene. To date this information has been collected by general databases such as OMIM or HGMD that collect only published mutations and in the case of OMIM selected published mutations. Unpublished mutations have made their way into Locus Specific Databases (LSDBs) and these can often contain 50% more mutations than published ones as well as other more detailed gene specific information. LSDBs however do not exist for all genes at this time.

The HUGO Mutation Database Initiative has addressed many problems regarding these issues and at the ASHG 2001 meeting a new society was inaugurated: the Human Genome Variation Society. This society aims to foster discovery and characterization of genomic variations including population distribution and phenotypic associations and promote the collection, documentation and free distribution of genomic variation information and associated clinical variations as well as endeavor to foster the development of the necessary methodology and informatics. The HGVS members have a number of projects, our main one being a new central variation database that will ensure capture of a maximum number of mutations, quality control of data submissions, safe storage and free-of-charge display via the Internet.

We outline the problems with mutation collection, why the current system cannot keep up and propose our solution as well as introduce our Society. **www.hgvs.org**.

Mutations in the *GJB2* gene among Bangladeshi with nonsyndromic hearing impairment. *J.H. Greinwald Jr*^{1,2}, *J.K. Bradshaw*², *L.H.Y Lim*¹, *M. Ali*³, *Y.S Guo*¹, *V. Pilipenko*¹, *D. Ingala*⁴, *M. Keddache*⁴, *D.I. Choo*^{1,2}, *R. Wenstrup*⁴, *R.J.H. Smith*⁵. 1) Center of Deafness and Hearing Research, Cincinnati, OH; 2) University of Cincinnati College of Medicine, Cincinnati, OH; 3) Dhaka Health Care, Dhaka, Bangladesh; 4) Dept of Human Genetics, Childrens Hospital Cincinnati, Cincinnati, OH; 5) Dept of Otolaryngology, University of Iowa, Iowa City, Iowa.

Background. Hereditary hearing impairment (HHI) accounts for approximately 50% of childhood hearing loss. Mutations in the *GJB2* gene are the most common cause of HHI in many countries. **Purpose.** We report the frequency of mutations in the *GJB2* gene in association with non-syndromic hearing impairment (NSHI) from Bangladesh. **Methods.** Fifty-eight patients with NSHI had DFNB1 testing. Thirty-one control subjects were identified with no sensorineural hearing impairment (HI). **Results.** Thirty-one of 58 (53.4%) had idiopathic severe to profound HI, 10 had moderate HI, and 3 had mild HI. Sequence variation was detected from direct sequencing from genomic DNA. One homozygous disease causing *GJB2* mutation (W77X) and one homozygote with a mutation of unknown significance (R127H) were identified in the Bangladesh NSHI population. Additionally several non-disease related polymorphisms were detected (V27I, E114G and V153I) including one control subject homozygous for V27I and heterozygous for E114G. Three affected and 5 control subjects were heterozygous for the R127H mutation. No carriers for known disease causing mutations were identified in the control or HI groups. **Conclusions.** The *GJB2* mutation rate in Bangladesh is considerably lower than previously identified in many other populations studied. These preliminary results indicate the need for further study of the genetic factors of NSHI in Bangladesh and throughout Southern Asia.

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MALDI-TOF mass spectrometry implementation for clinical molecular diagnostics. *I.M. Buyse, R.P. Pace, P. Lurix, B.B. Roa.* Baylor DNA Diagnostic Laboratory, Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

A major challenge for clinical molecular genetics is optimization of assay sensitivity/specificity and accuracy. The increasing demand for genetic testing services also requires a quantum leap in testing efficiency and throughput, which is addressed by improved technologies. Along these lines, our laboratory is converting DNA tests for known point mutations from the standard allele-specific oligonucleotide hybridization analysis (ASO) to a matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry platform (Sequenom). This PCR-based assay employs primer-extension using specific combinations of unlabelled dNTP/ddNTP mixes, followed by discrimination of the predicted extension products using mass spectrometry. This study involves the development, validation, and implementation of MALDI-TOF assays for new and existing tests. These include an Ashkenazic disease screening panel for common mutations in Tay-Sachs disease, Canavan disease, and cystic fibrosis (CF). Similarly, Ashkenazi mutation panels for Gaucher disease, Bloom syndrome, Familial Dysautonomia and Niemann-Pick Type A disease have also been developed. MALDI-TOF assays are in the process of implementation for cystic fibrosis, using an augmented CF panel for diagnostic testing and pan-ethnic carrier screening that exceeds the current recommended core panel of CF mutations. Assay design is greatly facilitated by the provided software, and testing efficiency is substantially improved by assay multiplexing which typically involves the use of 3 to 5-plex assays. Pilot studies and validation data from our laboratory indicate an extremely high level of assay sensitivity/specificity and accuracy, coupled with a marked increase in throughput. Challenges encountered in the conversion process include process automation for a 384-well PCR format, and MALDI-TOF integration with our existing lab information system to provide for continuous sample tracking. Implementation of the MALDI-TOF platform will address current and future needs for high-throughput genotyping in a DNA diagnostic laboratory setting.

RPGR gene mutations are clustered at the 5'end of an evolutionary diverged coding minisatellite in exon ORF15.

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Mutations in the RPGR (retinitis pigmentosa GTPase regulator) gene are known to be causal for the majority of X-linked retinitis pigmentosa (xLRP). However, there was a striking discrepancy between the proportion of families linked to the RP3 locus and those having mutations in the RPGR gene. Most of the missing mutations were shown to be situated in a mutational hotspot in a novel alternatively spliced RPGR exon (ORF15). Screening 58 xLRP families we found RPGR gene mutations in 71% of families with definite X-linked inheritance, with a clustering of RPGR gene mutations at the 5'end of the most repetitive sequence of exon ORF15. The comparison of these results with the distribution of ORF15 mutations in two further comprehensive mutational screens in large xLRP cohorts, reveals a strong polarity of frameshift mutations towards the 5'end of the most repetitive sequence in exon ORF15 in all three populations investigated. Subsequent detailed computerized analysis of the human coding sequence of exon ORF15 and the orthologous sequences in mouse, dog, cattle and pufferfish show that the most repetitive sequence in exon ORF15 is an evolutionary diverged coding minisatellite. Polarity of frameshift mutations towards the 5'end and frequent in-frame alterations within the tandem repeat array are reminiscent of mutation events in non-coding human minisatellites and indicate increased germline instability. The tandem repetitive structure conserved across species puts this unusual coding sequence in the context of repeats of base oligomers as the primordial coding sequences and their vestiges in modern genes.

Fine mapping and prevalence of *FOXC1* and *PITX2* dosage alterations. *M.A. Lines¹, F. Mirzayans¹, O.J. Lehmann², M.A. Walter¹.* 1) Med Genetics / Ophthalmology, University of Alberta, Edmonton, AB, Canada; 2) Institute of Molecular Biology, London, UK.

Mutations and deletions of the eye-expressed transcription factors *FOXC1* and *PITX2*, as well as duplications of *FOXC1*, are each potential causes of anterior segment developmental anomalies including Axenfeld-Rieger (AR) malformations and related clinical disorders. While the pathologic significance of mutations of these genes has been well demonstrated, the mechanism and frequency of occurrence of *FOXC1* and *PITX2* dosage alterations remains unclear. We are currently conducting mapping and cohort experiments aimed at assessing the extent and prevalence of 6p25 and 4q25 chromosomal microdeletions/duplications. We present a detailed microsatellite-based map of distinct but tightly clustered 6p25 duplications within three extensive anterior segment dysgenesis (ASD) pedigrees, complemented by real-time PCR-based screening of a panel of 70 sporadic ASD patients for similar findings. Attempts are underway to characterise the boundaries of each duplicated region in order to determine if genomic features may suggest a mechanism for this event. *FOXC1*, in particular, maps to the 6p subtelomere. Subtelomeric regions are GC-poor, repeat-rich, and increasingly appreciated as hotspots of chromosomal rearrangement. *FOXC1* dosage alterations may therefore be more common causes of AR than is currently appreciated.

Azoospermia Factor Microdeletions in Cryptorchidism, Varicocele and Idiopathic Infertility. *R. Dada¹, N. Gupta², K. Kucheria¹*. 1) Genetic Division, Anatomy, AIIMS, New Delhi, 110029, Delhi, India; 2) Urology Department, Anatomy, AIIMS, New Delhi 110029. India.

Deletions of the Azoospermia Factor (AZF) on the long arm of Y chromosome are an important cause of male infertility. AZF is located on deletion interval 5 and 6 and is associated with spermatogenic failure and has been used to define three regions on Yq (AZFa, AZFb and AZFc) which are critical for spermatogenesis and are recurrently deleted in infertile males. This loci contain genes and gene families critical for germ cell development and differentiation and deletion of these loci cause spermatogenic arrest at different stages of germ cell development. Infertility affects 10-15% couples attempting pregnancy and in about half the cases sperm production is defective either qualitatively or quantitatively. One hundred and twentytwo infertile males with oligozoospermia and azoospermia were included in this study. Semen analysis was done in each case to determine the spermatogenic status- normospermic (>20million sperms/ml), oligozoospermic (<20million sperms/ml) or azoospermic (no sperms in the semen). They were subjected to detailed clinical examination, endocrinological and cytogenetic study. Thirty G-banded metaphases were analyzed in 122 infertile males and PCR microdeletion analysis was done in 70 cytogenetically normal cases. For this genomic DNA was extracted using peripheral blood. The STS primers tested in each case were sY84, sY86 (AZFa); sY127, sY134 (AZFb); sY254, sY255 (AZFc). PCR amplifications found to be negative were repeated at least 3 times to confirm the deletion of a given marker. The PCR products were analyzed on a 1.8% agarose gel. Eight of the seventy cases showed deletion of at least one of the STS markers. Review of literature has shown that the overall frequency of microdeletions varies from 1 to 55%. In the present study the frequency of microdeletion in was 11.4%. AZF microdeletions were seen in both idiopathic and in non- idiopathic cases with Cryptorchidism and varicocele.

High-throughput SNP genotyping to identify genetic modifiers of complex multigenic mechanisms underlying severe CF liver disease. *K.J. Friedman¹, S.C. Ling², A.H. Handler¹, M. Macek Jr³, J. Zielenski⁴, P.R. Durie⁴, L.M. Silverman¹, M.R. Knowles¹ and CF Liver Disease Consortium.* 1) Depts Pathology & Lab Medicine, & Medicine, Univ North Carolina, Chapel Hill, NC; 2) Royal Hosp for Sick Children, Glasgow, Scotland; 3) Dept Pediatrics, Charles Univ, Prague, Czech Republic; 4) Depts Molecular & Medical Genetics, Pediatrics, Univ of Toronto, and Program in Genetics & Genomic Biology & Program in Integrative Biology, Hosp for Sick Children, Toronto, Canada.

CFTR mutations have limited predictive value in forecasting disease severity in CF, as patients with identical genotypes may or may not develop severe CF liver disease (CFLD). Data have implicated mutations in the alpha-1-antitrypsin (PI) and mannose binding lectin (MBL2) genes as risk factors for CFLD. In truth more complex interactions may influence development of hepatic fibrosis. Our data suggest these interactions exist, as the risk conferred by PI gene mutations is compounded by a high expression promoter variant (T @ -509) in the transforming growth factor beta (TGFB1) gene (OR 3.2 95%CI 1.2-8.2 P=.01). Dual presence of PI & MBL2 mutations also confers increased risk for CFLD (OR 4.6 95%CI 1.2-16.9 P=.038). Combination of mutations/variants in all three genes confers the greatest risk (OR 11.4 95%CI 1.3-104 P=.01). Additional genes are likely to contribute to CFLD. Approx. 100 genes have been identified as candidate modifiers for this outcome. These include genes underlying other inherited liver disorders, or mediate fibrogenesis, inflammation, immune or oxidative stress response, metabolism of the ECM, or influence hepatocellular proliferation. We have identified 193 validated single nucleotide polymorphisms (SNPs) linked to 85 of these candidate modifier genes. Many are functional while others are anonymous, and each will permit detection of risk-associated haplotypes. High-throughput assays for these SNPs are underway. Aggressive international recruitment of more patients has identified ~400 CFLD patients likely to meet our criteria. We anticipate analysis of 95 CFLD patients and ~200 matched controls will shortly identify additional genes that modify risk for CFLD.

Variation of FMR1 mRNA levels by repeat size and X-inactivation pattern. *E.G. Allen, S.L. Sherman.* Department of Human Genetics, Emory University School of Medicine, Atlanta, GA.

The fragile X syndrome, a type of inherited mental retardation, is due to the silencing of the FMR1 X-linked gene as a result of an expansion of an unstable CGG repeat sequence located in the 5 untranslated region (UTR) of the gene. Once expanded to over 200 repeats, the FMR1 gene is hypermethylated and consequently no message is transcribed and no protein produced. There are essentially four allelic forms of the gene with respect to the repeat length and they are referred to as normal, intermediate, premutation and full mutation. The defined repeat lengths are 40 repeats, 41-60 repeats, 61-199 repeats and 200 repeats, respectively, although there is overlap at the boundaries of these definitions. Until recently, the unmethylated, long CGG repeat track found in premutation carriers was thought to have little phenotype consequence. Now there is convincing evidence that female premutation carriers have an increase risk for premature ovarian failure (POF) whereas full mutation carriers have the same risk as the general population. However, the increased risk does not appear to be correlated with the size of the premutation. Recent reports have indicated that FMR1 mRNA levels are increased among premutation carriers compared with non-carriers and suggest that increased mRNA levels may be involved in the risk for POF. Thus, we are studying a large population of intermediate and premutation carriers to characterize the distribution of mRNA levels by repeat size in order to better define this molecular correlate. The population consists of blood samples drawn from 15, 15 and 15 non-carrier, intermediate and premutation males, respectively, and 20, 25 and 25 non-carrier, intermediate and premutation females. All subjects have been assessed with a large battery of neuropsychological measures and, for females, with a reproductive history, hormone levels and X-inactivation pattern. A clear understanding of the variation in levels of FMR1 mRNA will potentially provide a more powerful molecular correlate. This can be used to test the hypothesis of a direct relationship between increased mRNA levels and the risk for clinical involvement.

No Genotype-Phenotype Correlation in Holt-Oram Syndrome. A. Brassington¹, S. Sung¹, R. Toydemir¹, T. Le¹, A. Roeder¹, L. Jorde¹, M. Bamshad^{1,2}. 1) Department of Human Genetics, University of Utah, Salt Lake City, UT; 2) Department of Pediatrics, University of Utah, Salt Lake City, UT.

TBX5 is one of a family of genes encoding transcription factors that share a unique DNA-binding domain called the T-box. Mutations in *TBX5* cause malformations of the heart and upper limbs in Holt-Oram syndrome (HOS). It has been suggested that mutations in the 5' end of the T-box are associated with severe heart but mild limb defects, while mutations in the 3' end of the T-box cause mild heart and severe limb defects. We screened the entire coding region of *TBX5* in 45 families meeting the diagnostic criteria for HOS and discovered 15 mutations in 9 familial and 6 sporadic cases. Eight of these were missense mutations that cause amino acid substitutions in the T-box. Three missense mutations occurred at a previously described mutational hotspot (Arg237), and 3 were found at a newly described hotspot Thr233. Each of the missense mutations in the T-box is predicted to disrupt binding of TBX5 to its target site by changing an amino acid residue in direct contact with the target DNA or reducing the stability of the secondary structure required to maintain residues in a continuous DNA recognition surface. In 3 of 6 families with missense mutations in the T-box, both mild and severe defects of the heart and limbs were observed. Only 2 of 16 individuals with missense mutations in the T-box had the expected pattern of limb and heart defects, and in 7 of 16 individuals, we observed the converse of the expected pattern. Moreover, the spectrum and severity of malformations was similar between individuals with missense, nonsense, and frameshift mutations. We conclude that there is little evidence to support a genotype-phenotype correlation between mutations in *TBX5* and the variable expression of malformations in HOS.

Modifier genes in Cystic Fibrosis. *F. Belpinati*¹, *G. Malerba*¹, *C. Quinzii*¹, *C. Patuzzo*¹, *C. Bombieri*¹, *C. Castellani*², *B.M. Assael*², *P.F. Pignatti*¹. 1) Sec. Biology & Genetics, DMIBG, University of Verona, Verona, Italy; 2) Cystic Fibrosis Veneto Regional Centre, Hospital of Verona, Italy.

Cystic Fibrosis is an autosomal recessive disease caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. The severity and onset of clinical presentation in CF are highly variable, and the variability is explained only partially by allelic heterogeneity at the CFTR gene. Literature data suggest that the severity of pulmonary manifestation in CF may be correlated with other genetic factors. We have genotyped 83 CF patients attending the Veneto Regional CF Centre of Verona with defined genotypes carrying severe-severe CFTR gene mutations, for genes involved in inflammatory response (TNF α , LT α , FCeRIb), protease/antiprotease imbalance (a1AT, a1ACT) and host defense (MBL) candidate gene for disease modulation in CF. All the patients were clinically evaluated for respiratory parameters, gastrointestinal and nutritional status parameters, and other clinical variables. We have typed the following functional polymorphisms: TNF α -308; LT α NcoI; FCeRIb RsaI; a1AT A1237G, S, Z; a1ACT Thr-15Ala; MBL R52C, G54N and G57E. We found evidence for an association between TNF α -308 genotypes and distal occlusion obstruction syndrome ($p=0.0024$; OR=10, CI:1.0-460). The data also confirm the strong linkage disequilibrium between LT α NcoI* and TNF-3082* alleles ($p=0.00016$) as reported by the literature. A possible association between the R52C polymorphism of the MBL gene and multiresistant Staphylococcus infections was also detected ($p=0.0319$; OR=3.3, CI: 0.8-13.9).

Involvement of the *abca4* gene in autosomal recessive cone-rod dystrophies. *D. Ducroq¹, J.M. Rozet¹, S. Gerber¹, I. Perrault¹, F. Barbet¹, S. Hanein¹, S. Hakiki¹, J.L. Dufier², A. Munnich¹, C. Hamel³, J. Kaplan¹.* 1) INSERM U393, Hopital des Enfants Malades, Paris, France; 2) Service d'Ophtalmologie, Hopital des Enfants Malades, Paris, France; 3) INSERM U245, Montpellier, France.

In order to evaluate the implication of the ABCR gene in 16 autosomal recessive families and 13 sporadic cases of cone-rod dystrophy (CRD), 48 affected individuals were screened for mutations. All patients fulfilled the minimal criteria for diagnosis of CRD (ophthalmoscopic and electrophysiologic data). Using Denaturing High Pressure Liquid Chromatography (DHPLC) and direct sequencing techniques, we searched for mutations in the 50 exons and flanking intron sequences of the ABCA4 gene, in one affected patient per family. Sixteen different mutant alleles were identified in 12/29 patients. Among these 12 patients, 2 were homozygotes (2 consanguineous families), 4 were compound heterozygotes and 7 single heterozygotes. Among the 29 patients screened, 16 were affected with autosomal recessive CRD (ARCRD) and 13 were sporadic cases. ABCA4 mutations were found in 6/16 ARCRD (37.5%) and in 6/13 sporadic CRD (46.1%). The ABCA4 gene which has been identified in 1997 as the unique Stargardts (STGD) causing gene, has been later recognized as responsible for some forms of RP (RP19) and some CRD, depending on the nature of the ABCA4 mutations and on the remaining protein activity. Recently, A.Maugeri reported on the frequent implication of the ABCA4 mutations in patients affected with autosomal recessive CRD (65%) and speculated that Mutations in the ABCA4 (ABCR) gene are the major cause of autosomal recessive Cone-Rod Dystrophy. Considering that in all series, the screening of the ABCA4 gene failed to detect mutations in 20% of STGD patients, we made the assumption that the ABCA4 gene account for 46.9% of all autosomal recessive CRD and is indeed the major disease-causing gene in our series. In addition, we can calculate the risk that a sporadic case of CRD is an isolated form of autosomal recessive CRD when no mutation has been found in the ABCA4 gene. This risk is estimated to 10.5% according to a Bayesian calculation.

Novel insights in genotype-phenotype correlations in BPES through in-depth *FOXL2* mutation analysis. E. De Baere¹, D. Beysen¹, C. Oley², P. De Sutter¹, A. Garza³, C. Jonsrud⁴, P. Koivisto⁵, B. Leroy¹, B. Lorenz⁶, F. Meire¹, A. Plomp⁷, L. Van Maldergem⁸, A. De Paepe¹, J. Cocquet⁹, M. Fellous⁹, R. Veitia⁹, L. Messiaen¹. 1) Dpt of Med Genet, Gynaecol and Ophthalmol, Ghent University Hospital, Belgium; 2) Queensland Clin Genet, Royal Children's Hosp, Brisbane, Australia; 3) Grupo Trans-Americano de Esteril y Fertil, Mexico; 4) Univ Hosp of Tromsø, Dept of Med Genet, Norway; 5) Dept of Clin Genet, Tampere Univ Hosp, Finland; 6) Dpt of Ped Ophthalmol, Strabismol and Ophthalmogenet, Klin der Univ Regensburg, Germany; 7) AMC, Univ of Amsterdam, Clin Genet, The Netherlands; 8) Dpt of Hum Genet, Loverval, Belgium; 9) Hum Immunogenet, Inst Pasteur, Paris, France.

The blepharophimosis syndrome (BPES) is an autosomal dominant syndrome in which a complex eyelid malformation is associated (type I) or not (type II) with premature ovarian failure (POF). Both types have recently been ascribed to mutations in *FOXL2*, a forkhead transcription factor. We and others demonstrated a genotype-phenotype correlation: predicted truncated proteins lead to BPES type I, while particular predicted extended proteins cause BPES type II. In this study we extensively analyzed *FOXL2* in a series of unrelated BPES patients through (i) sequencing of ORF, 5' UTR and putative core promotor, (ii) Q-PCR, (iii) Southern blot (iv) FISH and (vi) microsatellite analysis of the *FOXL2* flanking regions. We have found 20 additional *FOXL2* mutations, most of which are novel. Upon revision of the genotype-phenotype correlation we found that: (i) the distinction between BPES type I and II is not so strict as previously stated as we observed the occurrence of an intermediate phenotype; (ii) the current genotype-phenotype correlation should be modified since we found exceptions against it in several families. As a consequence the predictive value of molecular testing concerning female infertility is still limited. Finally, in several well-established BPES families where no causal mutation was found, a position effect or mutations in another gene might be present and further attempts are underway to elucidate the defects in these families.

The contribution of genotypes at the MEFV and SAA1 loci to amyloidosis and disease severity in patients with Familial Mediterranean fever. *R. Gershoni-Baruch¹, R. Brik¹, M. Sinawi¹, M. Lidar², A. Livneh².* 1) Dept Human Genetics, Rambam Medical Ctr, Haifa, Israel; 2) Dept of Internal Medicine, Sheba Medical Center, Tel-Aviv, Israel.

Familial Mediterranean fever (FMF) is an autosomal recessive disorder characterized by recurring attacks of fever and serositis and predisposition to renal amyloidosis. Five sequence alterations (M694V, V726A, M680I, M694I and E148Q) in the pyrin/marenostrin (MEFV) gene account for the majority of FMF chromosomes. The wide clinical variability of the disease has been related to MEFV allelic heterogeneity. M694V homozygotes have a severe form of disease. Otherwise, a role for additional genetic and/or environmental has been proposed. Recently, polymorphisms at the SAA1 (serum amyloid A1) locus, or rather the SAA1/ genotype, were found to influence susceptibility to renal amyloidosis. We evaluated the contribution of genotypes at both the MEFV and the SAA1 loci to disease severity and amyloidosis. DNA samples from 274 FMF patients (152 males; 122 females), in whom two mutant FMF alleles have been identified, were further analyzed for genotypes at the SAA1 locus. The study group included 61 individuals with renal amyloidosis. We found that the SAA1/ genotype was strongly associated with renal amyloidosis but did not bear on disease severity (calculated using the Tel-Hashomer severity score). The majority of individuals with renal amyloidosis were homozygous for either the M694V or the complex V726A-148Q allele. The overall, male/female ratio was significantly higher among patients with amyloidosis than among patients without amyloidosis. The results of this study agree with the observation that the SAA1 locus plays a key role in conferring genetic susceptibility to amyloidosis. The contribution of male gender and genotypes at the MEFV locus to renal amyloidosis are equally significant.

Identification of modifying pathways involved in the phenotypic variability of spinal muscular atrophy (SMA). C. Helmken¹, Y. Hofmann¹, H. Raschke¹, S. Rudnik-Schöneborn², K. Zerres², B. Wirth¹. 1) Dept. Molecular Genetics, Institute of Human Genetics, Bonn; 2) Institute of Human Genetics, Aachen.

Proximal spinal muscular atrophy (SMA) is a neuromuscular disorder, caused by homozygous absence /mutations of the survival motor neuron gene 1 (SMN1). SMN1 is part of an 800 kDa protein complex ("gems") with a crucial role in snRNP biogenesis, pre-mRNA splicing and a presumable function in neural transport. SMN2, a nearly identical copy of SMN1, predominately produces exon 7-skipped transcripts, whereas SMN1 mainly produces full-length transcripts. The SR-like splicing factor Htra2-b1 was shown to interact with SMN and to restore full-length SMN2-mRNA to more than 80%. In rare cases siblings with identical 5q13-homologs and homozygous absence of SMN1 show variable phenotypes - affected or unaffected -, suggesting that SMA is modified by other, yet unknown factors. By analyzing seven such families, we demonstrated that in EBV-transformed cell lines homozygously deleted unaffected individuals not only display significantly higher levels of SMN protein, but also exhibit high expression levels of SMN-interacting proteins present in gems (Gemin2, Gemin3, and ZPR1) as compared with their affected siblings. In contrast, in primary fibroblast cultures no protein expression differences could be observed. Interestingly, other SMN-interacting proteins, which are not components of gems, such as p53, are not subject to a SMN-dependent regulation. Furthermore, Htra2-b1 was found to be up-regulated similarly as SMN. Since we could not find any sequence or transcription differences among intrafamilial siblings within SMN, Gemin2, Gemin3, ZPR1 or Htra2-b1, we postulate that the SMA phenotype is modified by a factor that influences the SMN complex, most probably acting upstream of Htra2-b1, which for its part up-regulates SMN, which then up-regulates other "gems"-complex members. Furthermore, this modulating effect is tissue-specific. The identification of the SMA modifying factor could represent an additional key-element on the way to develop a therapy in SMA patients based on the up-regulation of the SMN protein.

SMARD gene scan in patients with non-5q SMA. *A.Q. Hejmanowski¹, A.H.M. Burghes², T.W. Prior¹.* 1) Department of Pathology, Ohio State University, Columbus, OH; 2) Department of Molecular and Cellular Biochemistry, Ohio State University, Columbus, OH.

Spinal Muscular Atrophy with Respiratory Distress (SMARD) causes respiratory distress early in life and death occurs after the diaphragm is paralyzed. SMARD patients share some clinical features with SMA type I patients, including anterior horn cell loss, early age of onset, and short life expectancy. Recently, gene mutations have been identified in SMARD patients. We have thus begun screening our patient population with symptoms consistent with SMA who have been shown to be negative for the standard SMA deletion for SMARD related mutations.

Using fluorescent sequencing, a novel missense mutation, K220N, in exon 5, has been identified in 2 siblings. Although these patients do suffer from respiratory insufficiency and have distal muscle weakness, they have reached the ages of 9 and 11. Based on these initial findings, we feel that SMARD may exhibit a broader clinical phenotype, which includes patients with milder respiratory distress, and some shared SMA features.

It is imperative to determine whether a relationship exists between various gene mutations and disease severity. To that end, we are beginning a comprehensive DHPLC screening of the SMARD gene in our 410 SMA-like patients. To date, we have found 40 heterozygotes and 1 homozygote of a previously described SNP, S201L, also in exon 5, and numerous other polymorphisms throughout the gene.

Mutational spectrum of Pallister-Hall and Greig cephalopolysyndactyly syndromes. *J.J. Johnston, J. Turner, I.M. Olivos-Glander, L.G. Biesecker.* GDRB, NHGRI, NIH, Bethesda, MD.

Mutations in the zinc finger transcription factor GLI3, on chromosome 7p13, cause the Greig cephalopolysyndactyly (GCPS) and Pallister-Hall syndromes (PHS). PHS and GCPS are variable but distinct entities with numerous non-overlapping features. We hypothesized that GCPS is caused by functional haploinsufficiency of GLI3, while PHS is caused by the production of a truncated GLI3 protein that retains DNA binding activity. To test this hypothesis we analyzed patients with GCPS or PHS for GLI3 mutations. Individuals diagnosed with GCPS were first analyzed for deletions of the GLI3 locus. GCPS patients who were not deleted and all PHS patients were subjected to sequencing and/or dHPLC analysis to identify point mutations in GLI3. Our patient group consisted of 70 patients, 32 with GCPS and 38 with PHS. Eleven GCPS patients had large GLI3 deletions (>150 kb), leaving 59 patients to screen for mutations. A total of 34 mutations were identified and four additional patients have sequence alterations that predict amino acid substitutions that may be causative. Of the 22 mutations identified in PHS patients, all but one predicted a truncated protein 3' of the zinc finger domain and 5' to codon 1152 within the putative activation domains. These mutations fell in exons 13, 14 and the 5' end of exon 15. An additional PHS patient had a Pro>Arg alteration in exon 15 with no other alteration identified. Mutations in GCPS patients were more varied than those in PHS patients and occurred over a greater extent of the gene. No mutations were found in exons 13, 14, or the 5' end of exon 15 in GCPS patients. Alterations identified in GCPS patients included frame shift and stop mutations 5' to the zinc finger domain, frame shift and stop mutations at the 3' end of exon 15, in addition to the 11 large deletions. Three GCPS patients had sequence alterations of unknown significance. The distribution of mutations in these two phenotypes is significantly associated with the phenotypes ($p < .01$). These data support the hypothesis of a distinct pathogenetic mechanism for GCPS and PHS.

Snowflake vitreoretinal degeneration is genetically distinct from Stickler syndrome and Wagner disease. *M.M. Lee¹, C.J. Coulson¹, R. Ritter III¹, T. Hirose², A.O. Edwards^{1,3}*. 1) Department of Ophthalmology, UTSW Medical Center, Dallas, TX; 2) Schepens Eye Research Institute and Department of Ophthalmology, Harvard Medical School, Boston, MA; 3) McDermott Center for Human Growth and Development, UTSW Medical Center, Dallas, TX.

Purpose: To determine if Snowflake vitreoretinal degeneration is clinically and genetically distinct from Stickler syndrome and Wagner disease. **Methods:** Clinical data were collected on family members by history and examination. Thirteen family members underwent prospective examination and an additional twelve members were ascertained. Linkage analysis was performed with MLINK. **Results:** Six of the thirteen prospectively examined subjects had Snowflake vitreoretinal degeneration. Early onset cataract (5/6;83%), fibrillar vitreous degeneration (6/6;100%), and peripheral retinal abnormalities (5/6;83%) including minute crystalline deposits called snowflakes (4/6;67%) were common. Retinal detachment was seen in 1 of 6 (17%) of these prospectively examined subjects. A total of fourteen affected subjects were identified within the family and 3 (21%) had developed retinal detachment. Orofacial features such as cleft palate and midface hypoplasia and early onset hearing loss and arthritis typical for Stickler syndrome were absent. Linkage analysis excluded known vitreoretinal degeneration loci including the collagen 2A1 (*COL2A1*), collagen 11A1 (*COL11A1*), and Wagner disease loci. **Conclusions:** The absence of a retrolenticular membrane and presence of fibrillar vitreous degeneration were consistent with the vitreous structure reported for *COL11A1* and not *COL2A1* mutations. The absence of systemic features was characteristic of the vitreoretinopathies linked to chromosome 5q13 (e.g. Wagner disease) and mutations in exon 2 of the *COL2A1* gene. The absence of nyctalopia, posterior chorioretinal atrophy, and tractional retinal detachment were inconsistent with the chromosome 5q13 vitreoretinopathies. The clinical findings and linkage analysis demonstrate that Snowflake is a distinct vitreoretinal degeneration.

Phenotype and genotype characteristics of Korean patients with Gaucher Disease. *H.J. Kim, J.A. Yang, S.J. Park, I.S. Park, J.Y. Cho, G. Lee.* Dept Medical Genetics, Ajou Univ Col Medicine, Suwon, Korea.

Gaucher Disease (GD), a most common lysosomal storage disorder due to deficient activity of Glucocerebrosidase (GC), has been well known for its phenotypic variations and allelic heterogeneity. We found that non-neuronopathic form of GD (type1) to be rather aggressive with early childhood onset and rapid progression of massive organomegaly and skeletal complications leading to high morbidity and mortality, and high preponderance of neuronopathic(NP) form in Korean GD pts (more than 50%). To understand the molecular basis of the phenotypic characteristics of Korean GD, we performed mutational analysis of GC gene in 23 unrelated Korean GD pts (10 type1, 6 type2, 4 type3A, 3 type3B) by direct sequencing of PCR amplified genomic DNA and NciI restriction enzyme digestion methods. 15 different mutations were identified in 37 alleles; 10 alleles for L444P mutation, 4 alleles each for G46E, F213I and R257Q, 3 alleles for N188S and G202R, and 9 rare mutations of R48W, L160V, V191G, A232P, F331L, V375V, G377S, D409H and V460V were detected an allele for each. The spectrum of GC mutations found in Korean GD is rather unique compare to that of Jewish & Caucasian population. N370S, the most common GC mutation among Jewish (70%) and other Caucasian ethnic group (60-30%), is not found in Korean. The N370S is well established for its association with mild non-NP and the protective mutation from NP. Thus, the lack of N370S mutation among Korean may explain being severe type 1 and high preponderance of NP form (types 2 & 3). L444P, a common mutation (40%) among non-Jewish Caucasian and Japanese, is known to be associated with NP phenotype, esp. homoalleles in particular. However among 13 Korean NP pts, only 4 alleles (15%) and no homoalleles were detected. Among next common mutations, F213I, reported 14% in Japanese GD, was detected in both type I and NP pts, but G46E, never reported in Japanese GD, was detected only in type I pts, and G202R was only in NP. R257Q mutation previously known to be associated with mild phenotype, was detected both type1 and type2 pts. Comparison of the Korean GC mutations and phenotypes to that of Caucasian and Japanese will be presented.

Genotypic and phenotypic heterogeneity of DFNB1-related hearing impairment in the MidWestern US. *L.H.Y. Lim¹, J.K. Bradshaw^{1,2}, Y.S. Guo¹, V. Pilipenko¹, C. Madden¹, D. Ingala³, M. Keddache³, D.I. Choo^{1,2}, R. Wenstrup³, R.J.H. Smith⁴, J.H. Greinwald^{1,2}.* 1) Center for Hearing and Deafness Research, Children's Hospital Cincinnati, (CHMCC), Cincinnati, OH; 2) University of Cincinnati College of Medicine, Cincinnati, OH; 3) Department of Human Genetics, CHMCC, Cincinnati, OH; 4) Department of Otolaryngology, University of Iowa, Iowa.

Purpose. To determine the heterogeneity of, and correlation between the genotype and phenotype of DFNB1-related hearing impairment (HI) in a Midwestern USA population. **Methods.** Retrospective review of 160 consecutive children with idiopathic SNHL with DFNB1 testing. **Results.** The White: Black: Asian: Others distribution was 22: 1: 1:3. Correcting for 3 sib pairs, the prevalence of DFNB1 HI subjects having biallelic mutations was 15.3% (24/157). 37.5% (9/24) were homozygous 35delG, 25% (6/24) had other nonsense/nonsense mutations, and 37.5% (9/24) had a missense mutation in at least one allele. The allelic prevalence of 35delG was 8.6% (27/314) in the study population and 47.9% (23/48) in the DFNB1 group. The M34T allele mutation was next most prevalent at 2.2% (7/314) and 10.4% (5/48) respectively. Two novel mutations K15T and L90V were identified. Severe-profound HI occurred in 59.2% of DFNB1 subjects. Genotypes with biallelic nonsense mutations had a higher risk of severe-profound HI (88.2%). DFNB1 HI was usually bilateral, symmetric, non-progressive, and have flat audiograms. However, asymmetric HI (22.2%), sloping audiograms (25.9%), and even borderline-normal hearing in 1 ear was seen. These subjects were more likely to have at least 1 missense mutation. All 4 DFNB1 subjects with the M34T allele had mild HI in at least 1 ear. **Conclusions.** Our DFNB1-related HI, 35delG mutation and severe-profound HI rates were lower than previously described. Our missense mutation and M34T allelic prevalence were higher. Mild, asymmetric HI and sloping audiograms were more often associated with missense alleles. Two novel missense mutations coding for significant amino acid changes were found.

Characterization of a novel rhodopsin mutation (Pro170Arg) in a family with autosomal dominant retinitis pigmentosa (adRP). *D.K. Hughbanks-Wheaton¹, L.S. Sullivan², S.J. Bowne², S.P. Daiger², D.G. Birch¹.* 1) Retina Foundation of the S.W., Dallas, TX; 2) Human Genetics Center, Univ. Texas-Houston, Houston, TX.

Retinitis pigmentosa (RP) is a genetic eye disease characterized by progressive retinal degeneration. Degeneration typically occurs first in the peripheral retina, affecting the rod photoreceptors, and progresses inward toward the macula. This leads to restriction of the peripheral visual field (i.e., tunnel vision) and night blindness. RP is genetically heterogeneous and can be inherited in an autosomal dominant, autosomal recessive, X-linked recessive, or sporadic manner. Mutations in the rhodopsin gene are thought to account for approximately 30% of adRP cases. In an effort to identify mutations responsible for adRP we have identified a novel rhodopsin mutation. Using single-strand conformational polymorphism (SSCP) analysis followed by direct sequencing we identified a missense mutation at codon 170 resulting in a proline to arginine amino acid substitution within the 4th transmembrane domain of the rhodopsin gene. The residue at 170 is highly conserved in all vertebrates and mutations in this codon have not been described previously. In addition, this mutation has not been observed in 1,000 chromosomes tested. Affected family members (n=5) report first being aware of poor night vision in their late teens. Subjective peripheral field loss is first evident in the third decade. ERGs from four siblings aged 33-38 years revealed non-detectable rod responses in the two males. Both females retained rod a- and b- waves. The gain of rod photoreceptor transduction estimated from rod a-waves was reduced, suggesting that the mutant rhodopsin traffics to the outer segment and participates in the visual cycle. Cone b-waves were reduced in amplitude and delayed in implicit time in all patients with the mutation. The high conservation of the Pro170 residue, together with the cosegregation of this missense mutation with adRP in the affected family members supports the pathogenicity of this mutation.

Charcot-Marie-Tooth disease: A novel Tyr145Ser mutation in the Myelin Protein Zero (MPZ, P0) gene causes different phenotypes in homozygous and heterozygous carriers. *A. Leal^{1,2}, C. Kayser³, M. Berghoff⁴, E.*

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Mutations in the MPZ gene cause Charcot-Marie-Tooth type 1B (CMT1B), a demyelinating hereditary motor and sensory neuropathy (HMSN). Mutations in MPZ have been related also with axonal neuropathy (CMT2), particularly due to a T124M mutation in exon 3. Patients who carry this mutation are additionally affected with Argyll Robertson-like pupils and frequently with deafness and dysphagia. Here we present a Costa Rican family affected mainly with CMT1B; the parents presented a mild neuropathy with a late age of onset (50 years), whereas the two children presented an earlier age of onset (late 30 years) and a more severe neuropathy. Clinical signs are distal sensory deficits and absent ankle jerks. Pupillary abnormalities, distal weakness and atrophies are restricted to the children. Electrophysiological studies revealed axonal degeneration and demyelination. A sural nerve biopsy from one of the children showed signs of axonal degeneration as well as demyelination. Both in the maternal and the paternal family histories other patients with this type of peripheral neuropathy exist. After sequencing of all 6 coding MPZ exons for the parents and children, a Y145S mutation in exon 3 was found; the parents are heterozygous for this mutation, whereas the children are homozygous carriers. In contrast to the T124M mutation, only the homozygous Y145S carriers are affected with pupillary anomalies, and the Y145S heterozygous patients are less affected than the T124M heterozygous. We propose that by the exchange of similar side chains (uncharged polar) the slightly altered protein conformation has only low impact on the protein function; this could explain the mild phenotype of heterozygote carriers. However, a homozygous change could be sufficient to cause a neuropathy associated with pupillary anomalies.

Repeated Pregnancies Associated with Late Term Fetal Loss Malignant Ventricular Arrhythmias in a Mother Mosaic for an SCN5A Mutation. T. Miller¹, L. Baumbach¹, N. Moreno¹, E. Estrella¹, M.E. Ahearn¹, P. Rusconi¹, G. Wolff¹, P. Kurlansky², R. Myerburg¹, N. Bishopric¹. 1) Univ. of Miami School of Medicine, Miami, FL; 2) Miami Heart Research Institute, Miami, FL.

Germline mosaicism is a rare but important mechanism of genetic disease. Somatic mosaicism can explain some instances of reduced penetrance of disease-causing mutations, such as those detected in cardiac ion channel genes (*KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2*) associated with Long QT syndrome (LQTS). We investigated a 19-month African-American male with severe ventricular arrhythmias and his asymptomatic mother for LQTS. The child had fetal distress at 7 months gestation with *in utero* ventricular arrhythmia and postnatal *torsade des pointes* refractory to drug therapy, requiring delivery at 32 weeks and heart transplant at 6 months of age. He has had no further arrhythmias. His mother's previous pregnancy ended in late term stillbirth. She is asymptomatic, has normal EKGs, and denies consanguinity and has no known family history of heart disease, syncope or sudden death. We analyzed the child and his mother for mutations in all *KVLQT1* exons, and the most mutable *HERG* and *SCN5A* exons using exon-specific PCR, SSCP analysis, and direct DNA sequencing of SSCP variants. Importantly, we identified a heterozygous *SCN5A* mutation in the child, *R1623Q*, which has been previously reported as a *de novo* mutation in two unrelated Asian patients with LQTS. Initial sequence analysis of genomic DNA from the mother's peripheral blood suggested that she did not carry the mutant allele. We then employed an additional mutation detection technique that exploits the fact that *R1623Q* destroys a *HinfI* restriction site in *SCN5A*. We determined that ~ 10% of the mother's exon 28 PCR product was resistant to digestion. Sequencing of the undigested PCR product revealed exclusively the *R1623Q* allele, suggesting that the mother is a mosaic for this mutation. We are currently assessing the extent of somatic mosaicism in the mother. Our results suggest that maternal mosaicism for LQTS-associated mutations should be considered in cases of recurrent fetal loss and/or sudden infant death with normal parental phenotypes.

The effect of genetic modifiers on phenotypic variation in cystic fibrosis-associated pulmonary disease. *S.C. Hilmer¹, C.A. Merlo², R.A. Enke², M.E. Couch², E.P. Hoffman¹, J.G.N. Garcia², M.P. Boyle²*. 1) Ctr for Genetic Medicine, Children's Natl Medical Ctr, Washington, DC; 2) The Johns Hopkins Hospital, Baltimore, MD.

Cystic fibrosis (CF) is a lethal, multi-system autosomal recessive genetic disorder primarily affecting Caucasian populations. CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR), leading to the disruption of ion transport in epithelial lined organs and resulting in the plugging of ducts with obstinate, difficult-to-clear secretions. Over 1,000 mutations in CFTR have been identified, the most common of which is a deletion of a 3 base pair region resulting in the loss of a phenylalanine in the first nucleotide binding domain (DF508). Despite multisystem involvement, including lungs, sinuses, pancreas, bile ducts, sweat glands, intestines, bones and the reproductive tract, the majority of morbidity and mortality of CF patients is due to CF-associated lung disease. Recently, it has become apparent that CF patients with identical genotypes (specifically DF508 homozygotes) have considerable phenotypic variation, especially within the pulmonary system. We propose that this variation is the result of modifier genes that can be identified by use of oligonucleotide high-density microarray analysis of nasal epithelial cells. Gene expression analysis was performed using the Affymetrix GeneChip Human Genome U133 set on cells isolated from 15 CF patients with mild pulmonary disease, 10 patients with severe pulmonary disease, and 10 sex and age-matched controls. This technique allows for quantitative expression of 33,000 well-substantiated human genes. Genes involved with immune function, inflammation and transcription were found to be significantly up regulated in severe patients relative to mild. Additionally, 2 previously described CF phenotype modifiers (histone deacetylase and interleukin-8), were found to be differentially expressed in severe CF patients relative to mild patients. This data proves that candidate genetic modifiers can be identified through whole-genome expression profiling of small samples from primary scrapings of patient nasal epithelium.

Single nucleotide substitution in the COL1A1 gene (IVS13-10T®A) results in use of a new splice acceptor site, mRNA instability, and mild osteogenesis imperfecta. *I.A. Schafer^{1,2}, J. Stein², J.C. Hyland³, U. Schwarze⁴, P.H. Byers⁴, B.A. Clark².* 1) Metro Health Med Ctr, Cleveland, OH; 2) Cleveland Clinic Foundation, Cleveland, OH; 3) Center for Gene Therapy, Tulane University, New Orleans, LA; 4) University of Washington, Seattle, WA.

Osteogenesis imperfecta (OI) usually results from heterozygosity for mutations in the type I collagen genes, COL1A1 and COL1A2, which encode the pro α 1(I) and pro α 2(I) chains, respectively of type I procollagen. The analysis of the production of type I collagen by cultured skin fibroblasts is an adjunct to the identification of OI in children thought to have been abused. Cultured skin fibroblasts from a 27 day old girl who had a spiral fracture of the right femoral shaft and blue sclerae synthesized less than the normal amount of type I collagen but a definitive diagnosis of OI type I could not be established because informative markers that distinguished the two coding sequences were not found. The coding and exon-flanking sequences of the two type I collagen genes were screened by CSGE and a variant was identified in one COL1A1 allele (IVS13-10T®A) that introduced a new acceptor site at the -10, -9 positions of the intron and was not seen in 500 previously characterized alleles. If used, this would introduce an 8 nucleotide insertion that leads to a frameshift and creation of a premature termination codon in exon 25 of this 52 exon gene. RTPCR of RNA isolated from the cells identified a small amount of a fragment consistent with use of a new acceptor site. It was not clear if all transcripts from this allele used the new site or if some used the constitutive site. The 24 year old father and 61 year old paternal grandfather of the index patient both had blue sclerae, a history of fractures, and were heterozygous for the IVS13-10T®A mutation, consistent with the diagnosis of OI. These findings suggest that a spectrum of severity of bone fragility from normal to that seen in OI type I could be explained by "leaky" splice site mutations that lead to some normal splicing from the altered allele and titrate the amount of normal type I collagen in bone between control levels and those seen with OI type I.

Genotype/Phenotype Correlations in X-Linked Hypophosphatemic Rickets. *L.I. Valentine¹, T.J. Kirkpatrick¹, K.-S. Au¹, V.M. Rose¹, P.G. Brosnan^{1, 2}, B.W. Dominguez¹, D.A. Johnston³, H. Northrup^{1, 2}.* 1) Pediatrics, Univ of Tex Med School, Houston, TX; 2) Shriners Hospital for Children, Houston, Texas; 3) Univ of Texas MD Anderson Cancer Center, Houston, Texas.

X-linked hypophosphatemic rickets (XLH), a sex-linked dominant disorder of phosphate homeostasis, is the most common form of inherited rickets with an incidence of 1 in 20,000. XLH is characterized by defective renal phosphate handling and vitamin D metabolism leading to growth retardation, rachitic and osteomalacic bone disease, and hypophosphatemia. Conventional combined treatment of 1,25 dihydroxyvitamin D₃ (Rocaltrol) and inorganic phosphate salts has been well established to improve linear growth and heal rachitic skeletal abnormalities. Medication dosage is adjusted based on multiple laboratory parameters and linear growth. Inactivating mutations in the neutral endopeptidase gene, *PHEX*, are responsible for the XLH phenotype. We investigated whether type of *PHEX* mutation correlated with laboratory results and/or the development of therapy induced complications (nephrocalcinosis and hyperparathyroidism). Participants included 30 patients equally divided between Hispanics of Mexican descent (a previously unstudied group with XLH) and non-Hispanic Whites. Twenty different families were represented (20 individuals from 10 families with multiple affected individuals and 10 sporadically affected individuals). Eleven different mutations were detected with mutation identification in 80% of the multigenerational families and 50% of the sporadic cases. Multiple mutation types (missense, nonsense, splicing error, frameshift and deletion) were detected with seven novel mutations (not reported in the PHEXDB or medical literature). Mutation types were divided into two main types: missense and protein truncation. Ten patients with protein truncation mutations had a history of elevated PTH levels compared to only two patients with missense mutations. No correlation was observed with development of nephrocalcinosis. Testing additional patients for *PHEX* mutations and correlating with treatment regimen and laboratory values is needed to determine the significance of our genotype/phenotype observation.

***Rpp21* and *Rpp38* allele variants occur at different frequencies in severely and mildly affected CHH patients.** *M. Ridanpää*^{1,2}, *R. Fagerholm*^{1,2}, *I. Kaitila*^{2,3}, *O. Mäkitie*⁴. 1) Folkhälsan Institute of Genetics, Helsinki, Finland; 2) Department of Medical Genetics, University of Helsinki, Finland; 3) Department of Clinical Genetics, Helsinki University Hospital, Finland; 4) Hospital for Children and Adolescents, Helsinki University Hospital, Finland.

The RNase MRP has at least two functions required for cell growth, namely nucleolar cleaving of pre-rRNA and sequence-specific cleavage of RNA in mitochondrial DNA synthesis. All proteins and an RNA component are essential for the functional enzyme. We have recently described mutations in *RMRP*, encoding for the RNA component, in Cartilage-hair hypoplasia (CHH). A substitution A70G comprises 91% of the mutations in Finnish CHH patients and is the most common mutation worldwide. Despite of the same mutation in *RMRP* patients, even siblings at young age, can represent marked differences in the phenotype, indicating a role of genetic modifiers. We have studied three genes encoding for the proteins of RNase MRP in two Finnish CHH patient groups representing the most utmost phenotypes, all homozygous for A70G in *RMRP*. The 11 patients with severe phenotype had severe growth failure and complete alopecia (6) or severe hair hypoplasia (5). Six of them had hypoplastic anemia in infancy, 4 had Hirschsprung disease and 9 an increased susceptibility to infections. The patients with mild CHH had less severe growth failure and normal (2), mildly hypoplastic (6) or severely hypoplastic (1) hair. None of them had hypoplastic anemia, Hirschsprung disease or increased susceptibility to infections. In a search of polymorphisms in *Rpp20*, *Rpp21* and *Rpp38*, we found twelve base substitutions. In exon 2 of *Rpp21*, allele G was more common than allele A in severe CHH cases (12/20 vs. 7/16; 60% vs. 44%). Allele C in exon 2 of *Rpp38* was more common than allele G in severe cases (19/22 vs. 9/16; 86% vs. 56%). Both changes are predicted to cause an amino acid substitution. In addition, an intronic change of G for A in *Rpp38* was more common in severe than in mild cases (7/22 vs. 2/16; 32% vs. 13%). These results suggest that polymorphisms in the genes encoding for the protein components of RNase MRP may contribute to the phenotypic variability in CHH.

Dominant X-linked RP is frequently accounted for by truncating mutations in the exon ORF15 of the RPGR gene. *J.M. Rozet¹, I. Perrault¹, N. Gigarel¹, E. Souied¹, I. Ghazi², S. Gerber¹, J.L. Dufier², A. Munnich¹, J. Kaplan¹.* 1) INSERM U393, Hopital des Enfants Malades, Paris, CDX 15, France; 2) Service d'Ophtalmologie, Hopital des Enfants Malades, Paris, CDX 15, France.

In order to determine whether dominant X-linked retinitis pigmentosa (DXLRP), a condition previously described as different from the recessive XLRP (RP3), are due to mutations in the retinitis pigmentosa GTPase-regulator (RPGR) gene, we screened this gene for mutations in 14 families with severe expression in carrier females. Sequencing of the RPGR gene in affected individuals allowed the identification of 8 different null mutations in 9/14 families. All mutations were found to lie in the ORF15 exon. Interestingly, in 2/9 families, the mutation was found in an asymptomatic potential carrier. On the other hand, in-frame deletions or duplications reported earlier as polymorphisms were identified in 2/5 families with no mutation and direct sequencing of the RPGR exon ORF15 failed to detect any base change in the three remaining families. Nevertheless, linkage analyses confirmed the localisation of the gene in all families at the RP3 locus, suggesting that in these last 5 families, the disease might be caused by a mutation located in an unexplored region of the RPGR gene such as the promoter or the introns. In conclusion, we report here on the identification of null RPGR alleles in patients affected with DXLRP. Although, the age at onset of the disease in females is delayed compared to males (20-40 years versus 10-20 years, respectively), the visual impairment, the fundus alteration and the visual field reduction can be as severe in heterozygous females as in hemizygous males. In these females whose ERG is non-recordable, no preferential X-inactivation was observed. It would be extremely interesting to know the exact phenotype of females harbouring truncating mutations in the RPGR exon ORF15 in the XLRP families recently reported. Indeed, if some of the women were more severely affected than what is usually described for carrier females in recessive XLRP, we would have to consider RP3 as an incomplete dominant X-linked disease such as it is now reported for ornithine transcarbamylase deficiency.

Biochemical and genomic analysis of *ABCR* in STGD patients. *A.N. Yatsenko, N.F. Shroyer, R.A. Lewis, J.R. Lupski.*
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Stargardt disease (STGD1, OMIM#248200) is an autosomal recessive retinal dystrophy classically observed at an early age. The prevalence of STGD1 in the United States is estimated at 1 in 8,000 or less. The causative gene for STGD1 is located on chromosome 1p21 and has been cloned. This gene, *ABCR* (also called *ABCA4*), contains 50 exons and encodes a photoreceptor-specific ATP-binding cassette transporter. *ABCR* protein appears to function as an outwardly directed flippase for retinoids. Despite the confirmed linkage to the single *ABCR* locus, *ABCR* coding mutations account for only 66% - 80% of Stargardt disease chromosomes. The remaining 20-34% STGD alleles are unknown. We hypothesized a potential role for genomic rearrangements within *ABCR* region for these alleles. We have performed genomic Southern analysis for 100 STGD families with either one or no identified *ABCR* mutations. We found one deletion allele that eliminates *ABCR* exon 18 among the 200 chromosomes evaluated (~0.5%). STGD subjects with this deletion have a second mutant *ABCR* allele 2588G->C. The deletion spanning 1030 bp yields an in-frame deletion of 30 amino acids at positions 885-915 of the putative *ABCR* protein and is located at the linker region between transmembrane 1 and ATP-binding 1 domain, suggesting that this *ABCR* deletion is a disease-associated allele. Our breakpoint analysis of deletion region revealed a nonhomologous recombination with potential DNA topoisomerase I sites at both breakpoints. By deletion-specific PCR assay, we found the same allele deleted in 2 of 384 random controls (768 chromosomes, 0.26%) and 1 in 96 (~0.5%) AMD patients, but not in 96 age-matched controls with normal eye examinations or in 288 other STGD subjects. To understand the biochemical nature of this 1030 bp *ABCR* deletion, we created a plasmid construct with this *ABCR* alteration and performed transient transfection in 293 HEK cells. Biochemical analysis progresses. Our data indicate that genomic alterations of this type are a minor contribution to STGD mutant alleles. The remaining 20-34 % of Stargardt disease alleles remain unidentified.

Gene Expression in Inherited Human Craniosynostosis. *T.S. Zorick¹, L. Alonso², N. Alonso³, H. Matsushita⁴, M.R. Passos-Bueno¹.* 1) Biology, Univ Sao Paulo, Sao Paulo, Brazil; 2) Clinical Genetics, Escola Paulista Medicina, Sao Paulo, Brazil; 3) Dept of Plastic Surgery, Univ Sao Paulo, Sao Paulo, Brazil; 4) Dept of Neurosurgery, Univ Sao Paulo, Sao Paulo, Brazil.

Craniosynostoses are common congenital human birth defects, occurring 1 in 3000 live births. Craniosynostosis is caused by premature ossification of the cranial suture growth regions, which results in characteristic craniofacial abnormalities. Reconstructive surgeries are often necessary to alleviate the resultant clinical sequelae. Craniosynostoses are recognized as belonging to one of over 100 described genetic syndromes based upon the type of craniosynostosis observed, along with other dysmorphologies in other organ systems. About half of these are thought to have a monogenetic basis, which are generally autosomal dominant. The majority of the identified causative mutations have been found to map to genes for Fibroblast Growth Factor Receptors types 1-3. Although great progress has been made in recent years in molecular characterization of the primary genetic changes responsible for many cases of severe congenital craniosynostoses, the underlying mechanism for how these changes result in a particular disease phenotype remains largely unknown. The object of this research proposal is to identify genes that are differentially regulated in patients with Apert and Pfeiffer syndromes. We are collecting tissue samples isolated during corrective surgeries for patients with craniosynostoses, and culturing fibroblasts from these tissues. These cultured fibroblasts are then used to perform global analyses of gene expression on these cells in comparison to normal subject controls using SAGE (serial analysis of gene expression). Although many investigators have established clinical guidelines for grading the severity of the craniosynostoses, it is evident that the primary causative mutations are pleiotropic in their phenotypic expression. Therefore, an understanding of the downstream gene expression changes which occur among the various craniosynostosis syndromes will be much more informative and offers the promise of being able to improve clinical outcome prediction.

Epsilon-sarcoglycan mutations in three ethnically diverse families segregating Myoclonus-Dystonia. A.

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Myoclonus-dystonia (M-D) is an autosomal dominant disorder characterized by bilateral alcohol-sensitive myoclonic jerks involving arms and axial muscles. Dystonia, usually torticollis and/or writer's cramp, occurs in most patients and may occasionally be the only symptom. Patients often show prominent psychiatric abnormalities, including panic attacks and obsessive-compulsive behavior. Studies of M-D families demonstrated linkage to a locus on chromosome 7q21. Using positional cloning approach, Zimprich et al. (2001) have recently identified heterozygous loss-of-function mutations in the Epsilon-sarcoglycan (SGCE) gene. We evaluated 3 unrelated North- and South-American families with M-D for the presence of mutations by sequencing all eleven SGCE exons. In a family from Bolivia with early-onset M-D observed in four affected members, a G-to-A transition at nucleotide position 232 resulted in glycine-to-serine substitution at codon 78. A large North-American family of British descent with 12 known patients suffering from alcohol-sensitive M-D showed a G-to-A change at the highly conserved position +1 of the intron 1 splice donor site (IVS1+1G>A). Studies of functional consequences of this mutation are in progress. Finally, in an American family of German-Italian origin with five individuals affected with early-onset M-D, a mutation was detected at nucleotide 289 replacing C with T and resulting in termination of translation at codon 97. This latter mutation was previously described in a German M-D family.

A prevalent founder mutation among Ashkenazi Jews with Usher syndrome type 1. *T. Ben-Yosef¹, S.L. Noss², A.C. Madeo¹, A. Bar-Lev², J.H. Wolfman¹, Z.M. Ahmed¹, J.P. Willner², R.J. Desnick², K.B. Avraham³, H. Oster⁴, C. Oddoux⁴, A.J. Griffith¹, T.B. Friedman¹.* 1) Laboratory Molecular Genetics, NIDCD/NIH, Rockville, MD; 2) Department of Human Genetics, Mount Sinai School of Medicine, New York City, NY; 3) Department of Human Genetics and Molecular Medicine, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel; 4) Human Genetics Program, New York University School of Medicine, New York City, NY.

Usher syndrome is the most common cause of combined sensorineural deafness and blindness, and is a clinically heterogeneous disorder. The most severe form, Usher type I (USH1), is characterized by profound prelingual hearing loss, vestibular areflexia, and prepubertal onset of retinitis pigmentosa. Genes for six of twelve autosomal recessive USH loci have been identified. We studied the genetic basis of USH1 in the Ashkenazi Jewish population. Study subjects were 18 USH1 probands from 12 unrelated Ashkenazi families. DNA samples from four families were analyzed for the presence of shared haplotypes of genetic markers linked to six USH1 loci. A conserved haplotype and a homozygous recessive nonsense mutation were found for all affected individuals in these families. Of 12 unrelated USH1 probands, seven were homozygous for the nonsense mutation (58.3%); one was a compound heterozygote with a second putative mutation in the same gene (8.3%); and four were homozygous for the wild type allele (33.3%). Carrier frequencies of 0.79% (95% C.I. 0-1.8%) and 2.47% (95% C.I. 0.1-4.9 %) for the nonsense mutation were found among Ashkenazi Jews from New York and Israel, respectively. Based upon our findings, one prevalent nonsense mutation appears to account for the majority of USH1 cases in Ashkenazi Jews. The high carrier frequency of this mutation in this population predicts a prevalence of 0.15-1.5 affected persons per 10,000. Since the mutation was found in only 62 percent of USH1-bearing chromosomes from our Ashkenazi Jewish patients, the actual USH1 incidence risk is probably higher. The identification of this founder mutation will allow for better genetic counseling, carrier screening and molecular diagnosis, enabling hearing rehabilitation before loss of sight occurs.

Spontaneous mutations in CIAS1 gene cause neonatal onset multisystem inflammatory disease

(NOMID/CINCA). *I. Aksentjevich¹, F. Austin¹, J. Balow¹, J.J. Chae¹, E. Remmers¹, W.T. Watford², S.R. Hofmann², J.J. O'Shea², H. Komarow¹, N. Shoham¹, H. Hoffman⁴, R. Goldbach-Mansky³, D.L. Kastner¹.* 1) Genetics & Genomics Branch, NIAMS, NIH, Bethesda, MD; 2) Molecular Immunology and Inflammation Branch, NIAMS, NIH, Bethesda, MD; 3) Office of Clinical Director, NIAMS, NIH, Bethesda, MD; 4) Division of Rheumatology and Allergy/Immunology, University of California, San Diego, California.

Neonatal onset multisystem inflammatory disease (NOMID), also known as a chronic infantile neurological cutaneous and articular syndrome (CINCA), is a rare autoinflammatory disease characterized by skin rash, epiphyseal bony overgrowth, CNS involvement (chronic meningitis, sensorineuronal hearing loss, chorioretinitis), and other systemic features of inflammation. Most of the patients have presented as sporadic cases due to low reproductive fitness. We investigated whether patients with this disorder have mutations in the CIAS1 gene (encoding cryopyrin; PYPAF1; NALP3) which was recently identified as a gene causing the clinically similar Muckle-Wells syndrome (MWS). We have collected samples from 13 NOMID patients, and have identified 5 new missense substitutions: L264H, A374N, F523L c>a, F523L c>g, Y570C, and one mutation, D303N, which was recently reported by another group. None of these nucleotide changes were observed in any of over 700 control chromosomes, strongly supporting the hypothesis that they are mutations and not benign polymorphisms. In 3 patients where parental DNA was available, we showed that mutation arose de novo. Mutations in this gene have recently also been reported by others in seven additional European patients with NOMID /CINCA. Our findings reported here also rise the possibility of genetic heterogeneity, because 7/13 patients were negative for mutations, even after complete sequencing of CIAS1. Consistent with recent data linking cryopyrin to cytokine processing, monocytes bearing the D303N mutation showed increased level of IL-1 precursor by Western blotting. Peripheral blood mononuclear cells of this patient showed increased IL-3, IL-5, and IL-6, but not TGF(*)1 mRNA expression by Taq Man analysis.

Severe myoclonic epilepsy of infancy is caused by *de novo* mutations in *SCN1A*. *L.R.F. Claes, D. Audenaert, B. Ceulemans, K. Smets, A. Löfgren, J. Del-Favero, C. Van Broeckhoven, P. Dejonghe.* Department of Molecular Genetics, Flanders Interuniversity Institute for Biotechnology, University of Antwerp, Antwerpen, Belgium.

Severe myoclonic epilepsy of infancy (SMEI) is a rare disorder occurring in patients with no family history of a similar disorder. Early manifestations are tonic, clonic and tonic-clonic seizures in the first year of life. Seizures are often prolonged, generalized and associated with fever. Later in life, SMEI patients suffer from afebrile seizures. Early psychomotor and speech development is normal, but in the second year of life developmental stagnation occurs. Patients often become ataxic and speech development is delayed. There is no treatment that alters the patients outcome significantly. We screened the neuronal sodium channel α -subunit gene *SCN1A* in 16 unrelated SMEI patients and their parents and identified a single heterozygous *de novo* mutation in each patient: 4 frameshift, 3 nonsense, 2 splice donor and 7 missense mutations. For each patient paternity was confirmed, and none of the mutations occurred in 184 control chromosomes. All missense mutations were clustering in the S4-S6 region of *SCN1A* containing the channels voltage sensor and pore. There was no clear genotype/phenotype correlation since all SMEI patients had a severe phenotype independently of the type of mutation. Our data provide evidence that *de novo* mutations in *SCN1A* are the major cause of SMEI. Further, SMEI most likely results from *SCN1A* haploinsufficiency.

Phenotypic and genetic heterogeneity of autosomal recessive Limb Girdle Muscular Dystrophy (LGMD) in the Hutterite population. P.D. Frosk¹, A. Hoke³, K. Morgan², T.M. Fujiwara², C.R. Greenberg¹, K. Wrogemann¹. 1) Biochemistry and Med. Genetics, University of Manitoba, Winnipeg, MB; 2) Human Genetics and Medicine, McGill University, Montreal, PQ; 3) Clin. Neurosciences, University of Calgary, Calgary, AB.

Limb girdle muscular dystrophy is genetically heterogeneous and relatively common in the Hutterite population of North America with over 60 patients identified so far. Using samples from Hutterite families from Canada and the US we identified two loci for LGMD: *LGMD2H* (9q31-33) and *LGMD2I* (19q13.3). The putative disease-causing mutations occur in *TRIM32* (c.1459G>A, D487N) and *FKRP* (c.876C>A; L276I), respectively. To date these mutations account for all known cases of LGMD in this population. Therefore, we can provide accurate carrier testing and diagnosis for all the Hutterite patients with an LGMD phenotype. Given accurate molecular diagnosis, we have now begun to differentiate the two types of LGMD at the phenotypic level. Clinical differences are apparent between the two groups, with the *LGMD2I* patients having an earlier mean age at diagnosis, variable calf hypertrophy, a more severe course, and higher serum creatine kinase (CK) levels. Carriers of *LGMD2I* may have mildly elevated serum CK but are asymptomatic with respect to muscle weakness. Two of 15 *LGMD2I* patients have severe dilated cardiomyopathy and two others presented with reactions to general anesthetics. *LGMD2H* patients have lower serum CK levels, generally have a milder clinical course with respect to their myopathy, and none have shown muscle pseudohypertrophy nor have developed signs of cardiomyopathy or rhabdomyolysis. No patient homozygous for mutations in both *TRIM32* and *FKRP* has been identified although we have identified one patient homozygous for the *FKRP* mutation and heterozygous for the *TRIM32* mutation. This patient appears clinically indistinguishable from *LGMD2I* patients who are homozygous for the *FKRP* mutation and have no *TRIM32* mutation. These phenotypic observations will allow better clinical delineation and prospective management of Hutterite patients with LGMD and may help in our efforts to discern the pathogenesis in these two disorders.

Detection of mutations in the translation initiation factor eIF2B in a white matter disorder, the CACH/VWM

syndrome. *O. BOESPFLUG-TANGUY*¹, *A. FOGLI*¹, *E. EYMARD-PIERRE*¹, *D. RODRIGUEZ*², *E. BERTIN*³, *M. PINEDA*⁴, *R. SURTEES*⁵, *G. UZIEL*⁶, *E. MALASPINA*⁷, *R. SCHIFFMANN*⁸. 1) Fac de Medecine, Inserm U384, Clermont-Ferrand, France; 2) Hopital Trousseau, INSERM U546, Paris, France; 3) Dept of Neurosciences Bambino Gesu Children's Hospital, Rome, Italy; 4) Hospital San Joan de Deu, Barcelona, Spain; 5) Institute of Child Health, University College, London, United Kingdom; 6) Carlo Basta Institute, Milano, Italy; 7) Istituto di Clinica Padiatrica, Bologna, Italy; 8) National Institutes of Health, Bethesda, USA.

The Childhood Ataxia with diffuse Central nervous system Hypomyelination (CACH) or Vanishing White Matter (VWM) syndrome is an autosomal recessive leukodystrophy characterized by a progressive spastic ataxia with episodes of rapid deterioration following febrile infection or head trauma. Age of onset and disease severity are variable. MRI shows a diffuse abnormality of the cerebral white matter with an increasing number of cavitations. Genetic heterogeneity of this syndrome was recently confirmed by the description of mutations in the five subunits of the eIF2B translation initiation factor (alpha, beta, gamma, delta, epsilon). We analyzed the five EIF2B genes in 33 CACH families by genomic or cDNA sequencing. Missense mutations were detected in the EIF2B5 gene in 74% of families (in exons 3(60%), 2 (20%), 7 and 4 (10% each)). 52% of patients carried a R113H mutation in exon 3. Patients homozygote for this mutation express a mild form of the disease. Patients heterozygote composite with a R113H mutation have a milder phenotype than those without this mutation. Mutations in the beta, gamma, delta subunits of the eIF2B were detected respectively in 10% 8% and 10% of families. Mutation in the alpha subunit was found in only one family. In conclusion, EIF2B mutation represents a major genetic marker for the diagnosis of CACH/VWM, easy to detect in more than 50% of patient by testing the presence of the R113H mutation. Work is in progress to determine the consequences of these eIF2B mutations on the protective cellular mechanisms that respond to heat stress.

Identification of the Hydrolethalus Syndrome Gene on 11q23-25. *L. Lanyi*¹, *S. Finnila*¹, *I. Visapaa*^{1,2}, *J. Lee*¹, *R. Salonen*³, *L. Peltonen*^{1,2}. 1) Dept. of Human Genetics, David Geffen School of Medicine, UCLA, Los Angeles, CA; 2) Dept. of Molecular Medicine, National Public Health Institute and Dept. of Medical Genetics, University of Helsinki, Finland; 3) Dept. of Obstetrics and Gynecology, Helsinki University Central Hospital.

Hydrolethalus Syndrome (HLS) is a recessive lethal malformation syndrome characterized predominantly by hydrocephaly with absent midline structures of the brain. The disease is enriched in the Finnish population with an incidence of 1:20,000. We mapped the HLS 11q23-25 by linkage analysis and restricted the region to a critical 1cM interval using linkage disequilibrium (LD) and shared ancestral haplotype analysis. The coding region of all known genes and predicted transcripts on the critical region were sequenced. LD and haplotype analysis of informative SNP markers on the coding regions of the ten candidate genes further restricted the locus to 600 kb between markers D11S4258 and D11S975. A nucleotide variant was identified in a predicted transcript (Celera:hCG1644899) and showed complete association and cosegregation with the disease alleles. This variant is located in the coding region of a novel, predicted transcript with no known functional domains. The A to G mutation results in Asp221Gly change in the predicted 299 amino acid polypeptide. Mutation analysis in HLS families and 400 control DNA samples collected from Middle and Eastern Finland revealed that all affected individuals are homozygous for this variant and that the observed carrier frequency well agrees with the expected carrier frequency of 0.02 in these regions of Finland. No carriers were found in the European control sample of 200 chromosomes. The presence of the steady state transcript of this predicted gene was confirmed with Northern and RT-PCR analyses. The HLS gene is expressed and alternatively spliced in various tissues, transcript level being highest in brain and spleen. Northern analyses revealed no significant changes in the steady state transcript level in available tissues from affected individuals. Ongoing analyses are targeted to characterize the cellular and tissue localization of the HLS transcript and polypeptide.

The Expanding World of Molecular Mechanisms Involved in the Pathogenesis of Limb-Girdle Muscular Dystrophies. *V. Nigro*^{1, 2, 3}, *S. Aurino*^{1, 3}, *A. Belsito*^{1, 3}, *F. D'Amico*¹, *V. Ventriglia*¹, *M. Dionisi*³, *M. Esposito*³, *E. Marra*³, *V. Saccone*³, *G. Piluso*¹. 1) Dipartimento di Patologia Generale, Seconda Universita' degli Studi di Napoli, S. Andrea delle Dame, 80138 Napoli, Italy; 2) Centro di Eccellenza per le Malattie Cardiovascolari, Seconda Universita' degli Studi di Napoli, S. Andrea delle Dame, 80138 Napoli, Italy; 3) Telethon Institute of Genetics and Medicine (TIGEM), via P. Castellino 111, 80131 Napoli, Italy.

Limb-girdle muscular dystrophies (LGMDs) are progressive Mendelian disorders of proximal skeletal muscles. They are extremely heterogeneous both clinically and genetically. At least sixteen autosomal genes are involved in dominant and recessive forms. Our objectives are the identification of genes mutated in additional forms of LGMD and the identification of common pathways involved in the pathogenesis of muscle wasting. We assume that some of them can be found among the partners of gene products deficient in known LGMDs. Our strategy was: 1) bioinformatic scanning of the public and Celera databases by homology and electronic expression data. 2) Interaction screening of skeletal and cardiac muscle yeast two-hybrid cDNA libraries using the following bait cDNAs: dysferlin (LGMD2B), calpain-3 (LGMD2A), telethonin (LGMD2G), TRIM-32 (LGMD2H) and fukutin-related protein (LGMD2I) using each cDNA with causative missense mutations, as negative controls. 3) High-throughput mutation analysis, using DNA pools containing balanced mixes of two-three DNA from unrelated LGMD patients. These pools were PCR amplified for each exon of each gene and subjected to automated heteroduplex analysis by dHPLC (Transgenomic). We identified twenty putative LGMD candidate cDNA by yeast two-hybrid and several specific interactions were observed. By dHPLC we assigned patients to known LGMD loci and created a large pool of unassigned LGMD. We are identifying new genes possibly involved in the pathogenesis of LGMDs. This could improve both diagnosis and therapy approaches.

TDP1 mutations cause autosomal recessive spinocerebellar ataxia with axonal neuropathy and further illuminate the pathway for topoisomerase I dependent DNA damage repair. *H. Takashima*¹, *C.F. Boerkoel*¹, *J. John*^{1,2}, *G.M. Saifi*¹, *M.A.M. Salih*⁷, *D. Armstrong*⁶, *Y. Mao*⁴, *F.A. Quijcho*⁴, *B.B. Roa*^{1,3}, *M. Nakagawa*⁸, *D.W. Stockton*^{1,2}, *J.R. Lupski*^{1,5}. 1) Mole Human Gen.; 2) Kleberg Genotyp Ctr.; 3) DNA Diag. Lab.; 4) Howard Hughes MI, Baylor Col Med; 5) Pediatrics.; 6) Pathology, Texas Child Hosp, Houston, TX; 7) Pediatrics, Col Med, Riyadh, Saudi Arabia; 8) Third Dept Int Med, Kagoshima Univ, Japan.

Tyrosyl-DNA phosphodiesterase 1 (Tdp1) repairs covalent topoisomerase I (Topo I) DNA complexes and is essential for preventing the formation of double strand breaks resulting from the interference of stalled Topo I complexes with DNA replication in yeast. Surprisingly, we find that deficiency of such DNA repair in humans does not predispose to neoplasia or dysfunction of rapidly replicating tissues, but rather causes spinocerebellar ataxia with axonal neuropathy (SCAN1) apparently by affecting large terminally differentiated essentially non-dividing neuronal cells. Using genome-wide linkage mapping and a positional candidate approach in a Saudi Arabian family segregating autosomal recessive SCAN1, we identified a homozygous mutation in TDP1 (1478A>G, H493R). The His493 residue is conserved across species, and resides in the enzyme active site. Mutation of this amino acid to arginine predicts disruption of the symmetric active site structure by protein modeling. We suggest that loss-of-function mutations in TDP1 could cause SCAN1 either by interfering with DNA transcription or by inducing apoptosis in post-mitotic neurons. Intriguingly, the repair of Topo I induced DNA damage involves a coordinated biochemical reaction requiring the following enzymes: a proteolysis enzyme, Tdp1, the single-strand break repair complex (SSBR) [PNKP, DNA polymerase, DNA ligase III, XRCC1]. Aprataxin, causes early-onset ataxia with ocular motor apraxia and hypoalbuminemia (EAOH) when mutated, has been postulated to interact with SSBR. The similarity in phenotype between SCAN1 and EAOH could arise either because accumulation of DNA breaks gives rise to a common phenotype or because aprataxin acts in the same pathway as Tdp1 and is involved in the repair of Topo I induced DNA breaks.

Mandibuloacral dysplasia is caused by R527H mutation in lamins A/C gene. *F. Sangiuolo¹, G. Novelli¹, A. Muchir², A. Helbling-Leclerc², M.R. D'Apice¹, C. Massart², F. Capon¹, A. Botta¹, F. Amati¹, P. Sbraccia³, M. Federici³, R. Lauro³, C. Tudisco⁴, R. Pallotta⁵, G. Scarano⁶, B. Dallapiccola⁷, L. Merlini⁸, G. Bonne².* 1) Dept Biopathology Tor Vergata Univ, Rome,Italy; 2) INSERM U523, Inst de Myologie, Gr. Hospitalier Pitie-Salpetriere Paris,France; 3) Dept Int. Medicine Tor Vergata Univ,Rome,Italy; 4) Dept of Surgery Tor Vergata Univ,Rome,Italy; 5) Dept of Medicine,Univ Chieti,Italy; 6) Med.Genet.Division,Hosp.Benevento, Italy; 7) Dept Exper. Medicine Univ La Sapienza and CSS-Mendel,Rome,Italy; 8) Neuromuscular Unit,Rizzoli Inst, Bologna,Italy.

Mandibuloacral Dysplasia (MAD;MIM 248370)is a rare autosomal recessive disorder characterized by craniofacial anomalies,skeletal malformations and mottled cutaneous pigmentation.Through a homozygosity mapping we demonstrated linkage of MAD to chromosome 1q21 in 5 consanguineous Italian families.All patients shared a common haplotype(from markers D1S2715 to D1S2721),absent in 96 healthy controls.Multipoint analysis identified in this interval a maximum LOD score of 9.05.We identified in this region as candidate genes the lamins A/C(LMNA)on the basis of its involvement in Dunnigan-type familial partial lipodystrophy(FPLD)showing a partial overlapping phenotype with MAD.We sequenced LMNA gene and identified in all 9 patients the same homozygous transition, 1580GA (R527H).The mutation was absent in 300 unaffected individuals.In order to confirm the pathogenic effect of R527H mutation in MAD, we have analyzed cultured skin fibroblasts from a patient,using lamin specific antibody.MAD cells present lobulation of their nuclear envelope and abnormal lamin distribution.The R527 amino acid is located at external surface of C-terminal domain.Thus, R527H substitution would disrupt the surface structure of the protein altering binding fundamental sites. LMNA mutations cause other five distinct diseases:AD/AR Emery-Dreifuss muscular dystrophy,limb-girdle muscular dystrophy 1B,dilated cardiomyopathy type 1A,AR Charcot-Marie-Tooth disease type 2,and FPLD.This observation expand our knowledge on laminopathies and provide evidence for a wide range of effect of this protein on human development and differentiation.

The human homologue of the mouse *zfh-4* gene is a candidate gene for congenital bilateral isolated ptosis. *D.O.*

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Ptosis (blepharoptosis) is the abnormal drooping of the upper eyelid and can impair full visual acuity. Two linkage loci have been reported for congenital bilateral isolated ptosis, an autosomal dominant locus at 1p32-p34.1 and an X-linked dominant locus at Xq24-Xq27.1. A patient with isolated ptosis was identified with a de novo balanced translocation, 46, XY, t(1;8) (p34.3;q21.12). Sequence analysis of the translocation breakpoints showed that the chromosome 1 breakpoint does not disrupt a gene, however the chromosome 8 breakpoint interrupts a human homologue of the mouse *zfh-4* gene, encoding a zinc finger homeodomain transcription factor. Human ZFH-4 consists of 10 exons spanning ~161kb of DNA and the translocation breakpoint occurs in intron 4 resulting in truncation of the gene and its protein product. In mice it is known that *zfh-4* is expressed in neurones in the mid and hind-brain during embryogenesis. It is therefore possible that reduction in expression may affect the development of the oculomotor nuclei situated in the midbrain. The oculomotor nerves could be affected including the innervation of the levator palpebrae superioris (LPS), which is defective in inherited ptosis in humans. To determine whether ZFH-4 is truly associated with ptosis, we are currently investigating a cohort of patients with congenital bilateral isolated ptosis in an attempt to identify additional ZFH-4 mutations.

Identification and molecular characterization of a ring chromosome 18 associated with a vascular disease. A.A.

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Klippel-Trénaunay Syndrome (KTS) is a congenital vascular disorder comprised of capillary, venous and lymphatic malformations associated with overgrowth of affected tissues. In this study, we report the identification of a *de novo* ring marker chromosome in a patient with a vascular disease with some features of KTS. The ring chromosome is derived from chromosome 18, and appears to be mosaic with 25% of cells containing it. Fluorescence *in situ* hybridization (FISH) technique was used to define the chromosome 18 breakpoints associated with the ring chromosome. The chromosome 18p breakpoint was localized between the markers *WI-9619* and *D18S1150*, and is estimated to be less than 8.7 cM to the centromere. The 18q breakpoint was localized between the markers *UniSTS:148508* and *UniSTS:154383* and estimated to be less than 2.3 cM to the centromere. We conclude that the r(18) contains the chromosome 18 centromere and its size is estimated to be less than 11 cM. These studies define a new cytogenetic abnormality associated with KTS, and establish a framework for finding a potential vascular gene located close to the chromosome 18 centromere.

Disruption of Troponin-Tropomyosin Complex Causes Distal Arthrogryposis. *S. Sung*¹, *A. Brassington*¹, *K. Simpson*¹, *L. Jorde*¹, *J. Carey*², *M. Bamshad*^{1,2,3}. 1) Department of Human Genetics, University of Utah, Salt Lake City, UT; 2) Department of Pediatrics, University of Utah, Salt Lake City, UT; 3) Shriners Hospitals for Children, Intermountain Unit, Salt Lake City, UT.

The distal arthrogryposes (DAs) are a group of disorders characterized by congenital contractures of the hands and feet. We discovered that variant Freeman-Sheldon syndrome (FSS), the most common DA, is caused by mutations in *TNNI2*. *TNNI2* encodes an isoform of troponin I (TnI) that is a component of the troponin-tropomyosin (Tc-Tm) complex specific to fast-twitch myofibers. TnI prevents muscle contraction by inhibiting actomyosin ATPase activity. Binding of Ca⁺² ions to the Tc-Tm complex reverses this inhibition and causes muscle contraction. Each of the mutations in *TNNI2* is predicted to disrupt the carboxy-terminal domain of TnI. In the cardiac isoform of TnI, perturbation of this domain diminishes the sensitivity of TnI to Ca⁺² ions, reduces contractility, and impairs movement. This appears to be caused by mutant TnI interacting with normal molecules of TnI or other thin filament proteins. Mutations in *TNNI2* may cause congenital contractures by a similar mechanism. Variant FSS is a genetically heterogeneous disorder. Thus, we sought additional candidate genes by examining the modifiers of mutant isoforms of TnI in *Drosophila*. One of these modifiers is tropomyosin, another component of the Tc-Tm complex. A human homologue of tropomyosin is encoded by *TMP2*, located in the critical interval of another form of DA, distal arthrogryposis type 1 (DA1). Subsequently, we discovered that DA1 is caused by substitution of a highly conserved amino acid residue in tropomyosin. These findings suggest that DAs, in general, may be caused by mutations in genes encoding proteins of the contractile apparatus specific to fast-twitch myofibers. This would be analogous to the observation that mutations in the genes encoding cardiac-specific isoforms of the Tc-Tm complex cause a cardiomyopathy. Our discoveries provide us with an opportunity to directly study the etiology and pathogenesis of multiple congenital contracture syndromes and to consider novel therapeutic interventions.

Lay Advocacy Owned Blood and Tissue Banks. *S.F. Terry^{1, 2}, V.H. Whittemore³, C.T. Driscoll⁴, E.W. Johnson⁵, M.E. Davidson¹, P.F. Terry^{6,2}.* 1) President, Genetic Alliance, Sharon, MA; 2) Executive Director, PXE International, Washington, DC; 3) Tuberous Sclerosis Alliance, MD; 4) NHGRI, Bethesda, MD; 5) Barrow Neurological Institute, Phoenix, AZ; 6) Genomic Health, Redwood City, CA.

International lay advocacy groups accelerate and focus research efforts. Some international advocacy organizations, including those falling under the umbrella of larger groups such as the Genetic Alliance, have created small, niche repositories of biological materials. These banks provide an invaluable asset to the research community by streamlining some of the most logistically difficult aspects of searching for a gene, mutations and subsequent phenotype genotype correlations. Lay advocacy groups provide the basis for the informed decision making process, accessing and collecting appropriate DNA samples, protecting patient confidentiality, recontacting/redrawing patients as needed, collecting epidemiological data and reporting progress, or the lack of it, to research participants, in a culturally sensitive manner. The organizations are also uniquely able to safeguard confidentiality by acting as both a bridge and a 'firewall' between researchers, participants, and other organizations. The lay advocacy groups facilitate research by requiring collaboration, refining the focus and reducing the number of overlapping projects. These groups and the communities that support them are empowered in the process and foster the global effort to find the causes and cures for these diseases. Lessons learned from these banking efforts, all born of advocacy, for different diseases, using different models, will be discussed.

Identification and Haplotype Analysis of Single Nucleotide Polymorphisms in AMPD1 Gene. *K. Toyama¹, H. Morisaki¹, Y. Kitamura², N. Kamatani², T. Morisaki^{1, 3}.* 1) Dept. Bioscience, NCVC Research Institute, Suita, Osaka, Japan; 2) Inst. Rheumatology, Tokyo Women's Medical University, Tokyo, Japan; 3) Dept. Molecular Pathophysiology, Osaka University School of Pharmaceutical Sciences, Osaka, Japan.

AMPD1 deficiency, which shows relatively mild clinical phenotypes, is one of the most common enzyme deficiency in Caucasian. In addition to hereditary metabolic myopathy, this enzyme defect has been reported to be associated with improved cardiovascular survival in patients with chronic heart failure and coronary artery disease. However, the common type of AMPD deficiency has not been found in certain population including Japanese. Recently, we have identified a compound heterozygote of AMPD1 deficiency in Japanese myopathic patient. These findings suggest that the common variants are restricted to some population, and possibly one haplotype would account for the ancestral AMPD1 deficiency. We have identified 35 polymorphisms in and around the AMPD1 gene, including newly identified nonsynonymous variations. One of the new AMPD1 mutants showed reduced activity and different characteristics. By using LD Support software, we defined the typical haplotypes of AMPD1 locus and identified the ancestral haplotype responsible for the most common AMPD1 variant.

***ROR1* gene is mutated in the dominant form of Robinow syndrome.** *A.M. Vianna-Morgante*¹, *H.G. Brunner*², *H. van Bokhoven*², *B. Hamel*², *J.F. Mazzeu*¹. 1) Department of Biology, Universidade de São Paulo, São Paulo, Brazil; 2) Department of Human Genetics, University Medical Center, Nijmegen, The Netherlands.

Robinow syndrome (RS) is characterized by facial dysmorphisms, mesomelic shortening and hypoplastic genitalia. Both dominant and recessive forms of RS have been described that can be distinguished by the presence, in the recessive form, of costovertebral segmentation defects. The gene mutated in the recessive form, *ROR2*, is mapped at 9q22 and codes for an orphan tyrosine kinase receptor. Another member of the orphan tyrosine kinase receptor family is *ROR1*. We have previously described a partial duplication of chromosome 1p in a patient with the dominant form. One of the breakpoints at 1p31 disrupted the *ROR1* gene. We considered this gene as a candidate for the dominant form of Robinow syndrome and started a trial for mutations in *ROR1* gene in 36 unrelated patients in which *ROR2* mutations had been excluded. Exons 8 and 10 were partially analyzed. SSCP analysis of exon 8, which codes for the cysteine-rich domain, revealed altered patterns in five patients. In another patient we detected an insertion of 90 bp in exon 10, affecting the tyrosine-kinase domain. The cysteine-rich and tyrosine-kinase domains are those most frequently mutated in *ROR2* gene in patients with the recessive form of the syndrome. These results indicate that mutations in *ROR1* gene cause the dominant form of Robinow syndrome.

A silent coding change altering splicing of ATR, a DNA repair gene, is a cause of Seckel syndrome. *V. Ruiz-Perez¹, M. O'Driscoll², P. Jeggo², J. Goodship¹*. 1) Institute of Human Genetics, University of Newcastle upon Tyne, UK; 2) Genome damage and stability unit, University of Sussex, UK.

We have previously reported autozygosity mapping of a Seckel syndrome [MIM210600] locus to chromosome 3q22.1-q24. Additional family members enabled us to reduce the region of homozygosity and a candidate gene, ATR, remained within the region. Mutations were sought by cDNA amplification and direct sequencing. We observed skipping of exon 8 in an affected individual, introducing a frameshift and stop codon immediately after exon 7. Further cDNA analysis revealed a very low frequency of exon 8 skipping in normal controls in addition to the expected product. In the affected individual the proportions of the two products are inverted and two additional intermediate faint bands were seen. Sequencing these intermediate bands showed use of two alternative splice donors in exon 8. Genomic sequence analysis identified a silent single base change in exon 8. Wild type and mutant HIV-ATR hybrid minigenes were transfected into cos-7 cells. Analysis of the cDNA revealed two products with the wild type, corresponding to normal splicing and exon 8 skipping but found only the shorter allele with the mutant sequence. Hence splicing of exon 8 seems to be leaky in normal individuals and this silent base change has further decreased splicing efficiency. Ataxia telangiectasia related protein (ATR) and ataxia telangiectasia mutated (ATM) are related phosphatidylinositol 3-kinase-like kinases (PIKKs). ATM phosphorylates multiple substrates in response to DNA double strand breaks. ATR, in contrast, responds to other forms of DNA damage and to stalled replication forks. Cell lines derived from a patient from this family showed undetectable levels of ATR by western blotting. Additionally, we find that the phosphorylation of known ATR substrates is impaired in these cell lines. Whilst mutations in ATM cause ataxia telangiectasia, this represents the first report of patients defective in the ATR signalling pathway.

Angiotensin II Type 2 Receptor (AGTR2) Mutations Cause X-linked Mental Retardation. A.K. Srivastava^{1,2}, V.S. Vervoort¹, M.A. Beachem¹, P.S. Edwards¹, S. Ladd¹, K.E. Miller¹, X. de Mollerat^{1,2}, G.F. Guzauskas¹, K. Clarkson¹, C.E. Schwartz^{1,2}, B. DuPont^{1,2}, E. Boyd³, R.E. Stevenson^{1,2}. 1) J.C. Self Research Institute of Human Genetics, Greenwood Genetic Center, Greenwood, SC; 2) Department of Genetics & Biochemistry, Clemson University, Clemson, SC; 3) Fullerton Genetics Center, Asheville, NC.

X-linked mental retardation (XLMR) is the most common cause of inherited mental retardation in males. Linkage of several XLMR families to a large genetic interval, Xq23-q25, suggests the presence of one or more MR genes in the region. So far one MRX gene, the *PAK3* gene, has been identified within this interval. To identify other candidate MR genes, we analyzed a de novo balanced translocation [46, X, t(X;7)(q24;q22)] in a female patient with MR. We mapped the X chromosome breakpoint and analyzed the expression of the genes from the breakpoint region for functional nullisomy in the female patient with the translocation. We found that expression of the *AGTR2* gene in Xq24 is absent in this female patient. Additionally, we screened for mutations of the *AGTR2* gene in 5 probands from XLMR families putatively assigned to genetic intervals that encompass the *AGTR2* gene, in 33 probands from families with possible X-linked MR but no definitive linkage data, and in a large cohort of 644 unrelated male patients with MR of unknown causation but negative for Fragile-X. We identified eight mutations among these 682 MR males in the *AGTR2* gene including one frame-shift and three missense mutations. We also identified four polymorphic variants of the gene. The mammalian brain expresses two angiotensin II (Ang II) specific receptors, AGTR1 and AGTR2. Ang II actions on blood pressure regulation, water electrolyte balance, and hormone secretion are primarily mediated by AGTR1. The function of AGTR2 remains unclear. Our findings indicate a role for AGTR2 in brain development and cognitive function. If confirmed by further studies, this would indicate that mutations in the *AGTR2* gene is an important cause of mental retardation in males, accounting for 1% or more of mental retardation in males.

Adult-Onset Primary Open Angle Glaucoma (POAG) Caused by Mutations in Optineurin: A TNF-alpha-inducible protein that interacts with Huntingtin, RAB8, TFIIIA and E3-14.7K. *T. Rezaie*¹, *A. Child*², *R. Hitchings*³, *G. Brice*², *L. Miller*⁴, *M. Coca-Prados*⁵, *E. Héon*⁶, *T. Krupin*⁷, *R. Ritch*⁸, *D. Kreutzer*⁴, *R. Pitts Crick*⁹, *M. Sarfarazi*¹. 1) Molecular Ophthalmic Genetics Laboratory, University of Connecticut Health Center, Farmington, CT; 2) St. George's Medical School, London; 3) Moorfields Eye Hospital, London; 4) Pathology, UCONN Health Center, CT; 5) Ophthalmology and Visual Science, Yale University, CT; 6) Ophthalmology & Vision Research, Toronto, Canada; 7) University Eye Specialists, Chicago, IL; 8) New York Eye and Ear Infirmary, NY; 9) International Glaucoma Association, London.

We identified Optineurin (*OPTN*) as the mutated gene for adult-onset POAG, the *GLC1E* locus on 10p14. Screening of optineurin in 54 families with at least one low-pressure glaucoma subject identified one truncating (g.691insAG) and 2 missense (E50K and R545Q) mutations in 9 (16.7%) families. E50K was present in 7 families with 38 living glaucoma subjects, 18% of whom had high intraocular pressure. Another risk-associated change (M98K) was identified in 13.6% of glaucoma subjects and 2.1% of controls. Optineurin encodes for 577 a.a., contains 2 putative bZIP motifs, a C2H2-ZF type and several leucine-zipper domains. Two transcripts of 2.0 and 3.6-kb were present in trabecular meshwork (TM) and non-pigmented ciliary epithelium (NPCE). A 66-kDa protein was detected in TM, NPCE and aqueous humors of human and 7 other species. Immunocytochemistry showed a perinuclear localization of endogenous protein in the Golgi apparatus. Cloning of the mouse and monkey genes identified proteins with 584 and 571 a.a. and 78% and 96% identity to human *OPTN* respectively. Each of these 3 genes has 13 coding exons and their intron-exon boundaries are fully conserved. Optineurin shows similar intracellular localization in human, monkey and mouse. Immunohistochemistry identified optineurin in the anterior segments and optic nerves of human and monkey. Optineurin interacts with a number of proteins to regulate apoptosis, inflammation and vasoconstriction and it may have a neuroprotective role in glaucoma optic neuropathy. Supported By: EY-09947 and IGA.

Clinical and molecular characterisation of Frontotemporal dementia linked to Human Chromosome 3. G. Skibinski, L. Chakrabarti, and FReJA (Frontotemporal Research in Jutland Association). MRC Prion Unit, Institute of Neurology, London, UK.

Frontotemporal dementia linked to chromosome 3 (FTD-3) is an autosomal dominant pre-senile dementia. It is associated with a large Danish pedigree that spans 6 generations with over 1000 known individuals. Early onset (below 65 years) includes changes in personality, disinhibition, dyscalculia and hyperorality, progressing to a non fluent aphasia, speech disturbances and dystonic posturing. There is generalised cerebral atrophy with occasional frontal predominance and in one patient, a reduction in widespread cortical blood flow. Through haplotype analyses we have restricted the dementia locus to a 4cM region within chromosome 3. Using the current human genome sequence, candidate genes are being sequenced to identify any mutation that segregates with the disease, with particular attention being given to genes expressed in the brain. High-resolution FISH technology and Southern blot analysis are being used to identify genomic differences and chromosomal anomalies. Comparison of gene expression from FTD-3, Alzheimer and non-neurodegenerative brains will indicate aberrations in molecular pathways and complement the proteomic studies that are underway to investigate disruption to protein expression levels. In addition the development of an information extraction program will provide a novel in silico approach to obtain previously undiscovered molecular components and pathways from the literature relating to FTD-3.

In search of the forgotten exon of the human crumbs homolog 1 and its implication in LCA. *S. Gerber¹, I. Perrault¹, S. Hanein¹, S. Shalev², J. Zlotogora³, J.M. Rozet¹, F. Barbet¹, D. Ducroq¹, J.L. Dufier⁴, A. Munnich¹, J. Kaplan¹.* 1) Genetics, INSERM U393, Paris, France; 2) Genetic Unit, Haemek Medical Center, Afula, Israel; 3) Dpt of Community Genetics, Public Health Services, Ministry of Health, Ramat Gan, Israel; 4) Service d'Ophtalmologie, Hpital Necker - Enfants Malades, Paris, France.

In order to identify the gene responsible for Leber congenital amaurosis in a very large consanguineous family originating from Palestine. A genome wide search for homozygosity was undertaken in the nine affected patients of the family. The fluorescent oligonucleotides of the Genescan Linkage Mapping Set, version II (Perkin Elmer Cetus) were used. Amplified fragments were analysed on an automatic sequencer (ABI377). Genes lying in the candidate region were screened by direct sequencing in affected individuals. Novel CRB1 exons were searched by the screening of the Human Genome Working Draft and confirmed by RT-PCR of retinal mRNA. Homozygosity was found in several regions of the genome. Further analyses allowed to exclude these regions except one since homozygosity for the markers only reflected the lack of informativity in the parents. Conversely, we found evidence for homozygosity for markers of the 1q31 region. Recombination events in two affected individuals allowed us to reduce the genetic interval containing the disease-causing gene between D1S1723 and D1S2668. The CRB1 gene was found to map in this 4 cM interval. However, prior to this genome wide search for homozygosity, a screening of the six already known LCA-causing gene including CRB1 was performed and no mutation was found. The mapping of the disease-causing gene in this family prompted us to consider that the mutation might lie in a forgotten exon of the CRB1 gene. Subsequently, we screened the Human Genome Working Draft in order to identify novel CRB1 exons. This study revealed that a 12th exon lies in the 3'end of the gene. The sequence of this exon revealed that the disease in this family is accounted for by a homozygous 10 bp deletion leading to apparition of a premature Stop codon. Further study of this exon in 80 untreated LCA patients was therefore undertaken.

Novel mutations and genetic markers in Fabry disease. *K. Azibi^{1, 2}, C. Caillaud^{1, 2}, C. Heltianu³, J. Dussau¹, J.P. Puech¹, L. Poenaru^{1, 2}.* 1) Laboratoire de Genetique, Universite Paris V, CHU Cochin, Paris, France; 2) Institut Cochin, Inserm, CNRS, Paris, France; 3) Institute of Cellular Biology & Pathology N. Simionescu, Bucharest, Romania.

Fabry disease is an X-linked glycosphingolipid storage disease resulting from deficient activity of the lysosomal alpha-galactosidase A (GLA). This enzyme defect leads to the accumulation of globotriaosylceramide in the lysosomes of vascular endothelial and smooth muscle cells, but also in plasma. Therefore, clinical manifestations in the classical form are due to pathology of small vessels resulting in angiokeratoma, renal failure and heart and brain ischemia. Death occurs from renal or cardiac complications.

To clarify the molecular mechanism causing the enzyme defect, the alpha-galactosidase gene was amplified and sequenced for each 43 patients and 25 carriers from 59 families. 30 mutations (14 novel and 16 previously described) were identified. The novel mutations were : two nonsense mutations (W245X and W262X), five missense mutations (A156N, S297C, P265R, I354K and Q279H), a small nucleotide insertion (InsG10678) and six deletions (1235del15, 5113delA, 10593delAT, 7316delA, 7335delG and delATCA). Our study defines the previously reported heterogeneity of mutations causing Fabry disease, but no strict genotype-phenotype correlation was found. It has also permitted to clarify the carrier status in females and to offer them prenatal diagnosis.

Recently, polymorphisms of endothelial dysfunction markers genes, eNOS, angiotensin-converting enzyme (ACE), angiotensinogen (AGT) and the type 1 (AT1) and type 2 (AT2) angiotensin II receptors, have been considered to play a role in the pathophysiology of vascular, heart and renal diseases. Thus, alteration of the nitric oxide pathway has been previously shown in Fabry disease. Analysis of different genetic markers of the endothelium dysfunction may allow to determine the role in the clinical heterogeneity of Fabry disease.

Novel mutations in type B Niemann-Pick disease: Implications for phenotype/genotype correlations. *C.M.*

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Types A and B Niemann-Pick disease (NPD) result from deficient acid sphingomyelinase (ASM) activity. Type A NPD is characterized by neurodegeneration that leads to death by ~3 years of age, whereas patients with Type B NPD have little or no neurological involvement and may survive into adulthood. Enzyme replacement therapy has been evaluated in a mouse model of NPD, and markedly improves the visceral organ pathology. These studies provided "proof of principal" that this approach will be beneficial for Type B NPD patients, and a clinical trial is currently being planned. We have therefore been performing ASM genotype analysis on Type B NPD patients from around the world, including over 40 patients evaluated at Mount Sinai. Over 80 novel mutations have been identified in over 150 Type B NPD patients. Among these are several common mutations not previously reported, including a point mutation found in 10 patients of Scottish descent, three point mutations in 16 Type B patients from Saudi Arabia, and several point or frame-shift mutations found in patients of Turkish descent. We have also extended previous analyses of the common deltaR608 mutation, and found that throughout the United States, Brazil, and several countries in Western Europe it accounts for about 30% of the Type B NPD alleles. Phenotype analysis of the patients revealed that the Saudi mutations were associated with an aggressive, but non-neurological, form of Type B NPD, whereas the Scottish mutation was associated with a milder form. Continued analysis of the deltaR608 mutation confirms that this mutation is also associated with a less severe form of Type B NPD. These ongoing studies provide a basis for phenotype/genotype correlations in Type B NPD, and should aid in the selection of appropriate patients for the enzyme therapy clinical trials.

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A novel mutation in the X-linked form of Charcot-Marie-Tooth neuropathy. *G. Karadima, M. Panas, P. Floroskoufi, N. Kalfakis, D. Vassilopoulos.* Dept Neurology, Athens National University, Athens, Greece.

X-linked Charcot-Marie-Tooth disease (CMTX) is a sensorimotor peripheral neuropathy linked to mutations in the gene encoding connexin 32. In the present study DNA-based mutation analysis on the connexin 32 gene (Cx32) was performed in 5 patients with CMT type 1 phenotype but without duplication involving the chromosomal region 17p12-p11.2. The coding region of the connexin 32 gene, entirely contained in exon 2, was screened for mutations. Mutation screening was performed by SSCP analysis after PCR amplification of the coding region. Automated sequencer performed sequencing of the candidate fragments. We found 5 missense mutations of which 1 is new: Val38Ala. Restriction enzyme digestion was used to confirm the sequencing results of the new mutation. The mutant allele was absent in 100 healthy controls. The identification of this new CMTX causing mutation is a critical step for carrier detection and presymptomatic diagnosis.

Contribution of two X-linked loci, FRAXE and XNP to mental retardation in South Africa. *F.B. Essop, A. Krause.*
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Mental retardation (MR) affects 1-2% of the general population. About 50% of severe cases of MR have a genetic cause, and almost half of these are said to be due to X-linked mental retardation (XLMR). XLMR can be subdivided into syndromic XLMR (MRXS) and nonspecific XLMR (MRX). MR is usually a secondary feature in MRXS. MR is the only feature seen consistently in MRX. This study aims to investigate the contribution of two X-linked loci associated with XLMR, viz. the XNP gene and the FRAXE, FMR-2 gene to MR in South Africa, with a view to improving diagnostic services for MR. The contribution of the locus to MR in South African populations is currently being assessed by screening males with a range of MR- either from institutions or routine referrals for fragile X A syndrome testing who tested negative for the FRAXA mutation. FRAXE syndrome, the most commonly identified MRX gene, is associated with a mild form of MR. At least 255 MR individuals have been tested for the FRAXE mutation. Of these, 182 have tested negative for FRAXE and 73 samples are currently being retested. Mutations in XNP have been shown to be relatively commonly involved in severe XLMR. The zinc finger helicase-coding region of XNP is a hot spot for mutations. As mutations in the XNP gene lead to a severe MR phenotype, screening for XNP mutations in MR males who have a negative family history was suggested. At least 187 MR males have been screened for mutations in the hot spot region. These results are currently being analysed. If the two new markers are shown to contribute significantly to the causes of XLMR in the South African population, they will be implemented as new diagnostic tests.

The novel Rho-GTPase activating gene *MEGAP* has a putative role in severe mental retardation. V. Endris¹, B. Wogatzky¹, U. Leimer², D. Bartsch², M. Zatyka³, F. Latif³, E.R. Maher³, G. Tariverdian¹, G.A. Rappold¹. 1) University of Heidelberg, Institute of Human Genetics, Heidelberg, Germany; 2) Central Institute of Mental Health, Mannheim, Germany; 3) Section of Medical and Molecular Genetics, Department of Pediatrics and Child Health, University of Birmingham, Birmingham, UK.

In the last few years, several genes involved in X-specific mental retardation have been identified using genetic analysis. Although it is likely, that additional genes responsible for idiopathic mental retardation are also localized on the autosomes, cloning and characterisation of such genes have been elusive so far. Here we report the isolation of a novel gene, *MEGAP*, which is disrupted and functionally inactivated by a translocation breakpoint in a patient, who shares some characteristic clinical features, such as hypotonia and severe mental retardation, with the 3p- syndrome. By FISH and LOH analysis, we demonstrated that this gene resides on chromosome 3p25 and is deleted in 3p- patients. *MEGAP* mRNA is predominantly and highly expressed in fetal and adult brain and specifically in the neurons of the hippocampus and cortex, structures known to play a pivotal role in higher cognitive function, learning and memory. We describe several *MEGAP* transcript isoforms and show that *MEGAPa* and *b* represent functional GAP proteins by an in vitro GAP-assay. *MEGAP* has recently been shown to be part of the Slit-Robo pathway regulating neuronal migration and axonal branching, highlighting the important role of *MEGAP* in mental development. We propose that haploinsufficiency of *MEGAP* leads to abnormal development of neuronal structures that are important for normal cognitive function.

Identification of autosome-linked mental retardation (MR) mutations: mapping and characterization of balanced autosome;autosome translocations associated with MR. *M.A. Beachem, S. Ladd, V.S. Vervoort, L.R. Taylor, G.F. Guzauskas, R.J. Schroer, R.E. Stevenson, B. DuPont, A.K. Srivastava.* J.C. Self Research Institute of Human Genetics, Greenwood Genetic Center, Greenwood, SC.

Mental retardation (MR) is the most common developmental disability affecting about 2-3% of the human population. Genes that cause inherited MR are distributed throughout the human genome. In recent years several MR genes, especially on the X chromosome, have been isolated but success in identifying autosomal MR gene has been very limited. So far only few suggestive autosomal MR loci have been reported based on studies of a limited number of familial cases and studies of patients with MR and balanced chromosomal rearrangements. Extending the latter approach, we have characterized the chromosomal breakpoints in several patients with MR and autosome;autosome translocations. In principle, at least one of the two chromosome breakpoints is likely to disrupt or alter expression of a specific gene, thus producing the MR phenotype in these patients. We defined seven potential chromosomal regions on chromosome 3q12, 5q15, 6q22, 16q24, 17q23 and 21q22 potentially harboring candidate MR genes. In five patients, we have identified clones/markers flanking one of the two translocation breakpoints. We have identified the genomic contigs that span one of the two breakpoints in two patients. FISH and PFGE analyses have narrowed the critical breakpoint regions associated with MR to less than 1 Mb in five cases. One autosomal breakpoint mapped within the Down syndrome critical region on chromosome 21. Due to the lack of large familial autosomal cases with MR, which normally assist in defining linkage intervals associated with MR on the genome, the balanced translocations associated with MR analyzed here provide a most valuable resource for the mapping of MR loci. The mapping information presented here will be useful in the identification of the genes associated with human cognitive functions.

ARX mutations frequently cause X-linked mental retardation. *M.E. Mangelsdorf^{1,2}, M. Finnis¹, L. Hobson¹, B. Hodgson¹, G. Turner³, M. Partington³, C. Schwartz⁴, R.E. Stevenson⁴, H. Lubs⁵, E. Holinski-Feder⁶, J. Mulley^{1,7}, J. Géczy^{1,2}.* 1) Department of Cytogenetics and Molecular Genetics, Women's and Children's Hospital, Adelaide, Australia; 2) Department of Paediatrics, University of Adelaide, Adelaide, Australia; 3) Hunter Genetics and University of Newcastle, New South Wales, Waratah, Australia; 4) Greenwood Genetic Center, Greenwood, SC 29646, USA; 5) University of Miami School of Medicine, Miami, FL; 6) Medizinisch Genetisches Zentrum, Bayerstrasse 53, D-80335, Munchen, Germany; 7) Department of Molecular Biosciences, University of Adelaide, Adelaide.

We have recently described mutations in the *Aristaless*-related homeobox (*ARX*) gene which cause both syndromic and non-syndromic X-linked mental retardation (XLMR) (Strømme et al, 2002). Our initial studies showed that 9/11 families that mapped to Xp22, in which *ARX* is located, contained a mutation in this gene. One mutation (428-451 dup(28bp)), which results in expansion of a polyalanine tract (12-20 alanines) made up more than half of the mutations found. This duplication causes both non-syndromic as well as a variety of syndromic XLMR (X-linked infantile spasms syndrome [ISSX], Partington Syndrome). Haplotype analysis suggests that these mutations are separate events and are not due to a founder effect, and that this polyalanine tract represents a mutation hotspot within the gene. In the past genes implicated in non-syndromic MR have only been found in few of the families mapping to the region in which the gene resides. In order to determine the frequency of *ARX* mutations causing MR we have screened, by SSCA, (1) additional families which have been mapped to the region, (2) boys with familial MR suggesting X-linked inheritance but too small for linkage and (3) boys representing sporadic cases previously shown not to contain expansions at the *FRAXA* CCG repeat. Additional 428-451 dup(28bp) mutations were found in these groups. Apart from CCG expansions associated with *FMR1* and *FMR2*, only *ARX* accounts for a significant proportion of MR families mapping to a specific region of the X. Furthermore, *ARX* mutations may be present in some of the patients usually referred for fragile X syndrome testing.

Impaired fuel homeostasis leads to defective non-shivering thermogenesis in the 70kDa Peroxisomal Membrane Protein (PMP70) deficient mouse. *I. Silva-Zolezzi*^{1,2}, *K.J. Hebron*¹, *G. Thomas*³, *D.S. Millington*⁴, *V. Valle*¹, *G. Jimenez-Sanchez*¹.

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PMP70 is one of four known half ABC transporters in mammalian peroxisomes. To better understand its function and implications for human disease, we produced *PMP70* knockout mice (*PMP70*^{-/-}) by standard methods. These mice are viable and exhibit impaired peroxisomal β -oxidation of phytanic and pristanic acids and a variety of metabolic abnormalities including reduced hepatic glycogen in the fed state; a medium chain dicarboxylic aciduria that increases with fasting; and increased expression of peroxisomal proliferator-activated receptor alpha (PPAR α) target genes such as *MCAD*, *ACOX* and *ACAA1* in the liver. Surprisingly, the *PMP70*^{-/-} mice also have a defective non shivering thermogenesis (NST) as shown by a drop in body temperature to $<15^{\circ}\text{C}$ after 2-6h at 4°C (controls $35\pm 3^{\circ}\text{C}$), similar to that of mice with defects in mitochondrial β -oxidation (eg. *LCAD*-deficient mice). In contrast to these other models, *PMP70*^{-/-} mice have normal brown adipose tissue (BAT) lipid content and normal levels of BAT *UCP-1* expression. *PMP70*^{-/-} mice do however have a reduction in plasma carnitine (total carnitine 22.8% of controls). Moreover, blood glucose levels are normal in the fed state but are $\sim 50\%$ of control after a 4.5h cold exposure (99 ± 21 vs $193\pm 37\text{mg/dl}$). To study the role of fuel homeostasis in NST of *PMP70*^{-/-} mice, we determined cold tolerance before and after 10 days of a high carbohydrate (CHO) (70%) diet or 4 weeks of a carnitine-supplemented (0.5g/Kg/day) diet. Both diets delayed the onset of hypothermia from $\sim 3\text{-}4\text{h}$ to $\sim 9\text{-}11\text{h}$. Furthermore, after returning to a standard CHO diet for 2 weeks, *PMP70*^{-/-} mice again evidence a profound defect in NST. Similar studies with carnitine repletion are underway. Our results suggest that accumulation of phytanic and pristanic acids in the *PMP70*^{-/-} mouse activate PPAR α , leading to inappropriate activation of the fasting fuel homeostasis that may limit energy substrates leading to NST in this mouse.

RNA Instability in Carbamyl Phosphate I (CPS-1) Deficiency Resulting from Intronic and Exonic Mutations Results in Message Homozygosity for the Other Mutation in at Least One Third of Patients. *M.L. Summar^{1,2}, L. Hall¹, A. Payne², A. Willis².* 1) Dept Pediatrics/Div Genetics, Vanderbilt Univ Medical Ctr, Nashville, TN; 2) Molecular Physiology & Biophysics.

CPS-1 deficiency (CPS1D) is a rare autosomal recessive disorder which causes either a lethal or severe defect in the function of the urea cycle. As part of our mutation studies of CPS1D we have studied mRNA and Genomic DNA in all patients where both were available. We have noted that over 1/3 of patients analyzed for both tissues are homozygous for a missense or nonsense mutation in RT/PCR products of CPS-1 but heterozygous for these mutations in PCR products of genomic DNA. We hypothesized that the missing allele might be the result of genomic changes which result in an unstable transcript. We studied 24 patients for whom both RNA and DNA were available. We screened all 37 exons and sequence 5' and 3' of the transcript by PCR of genomic DNA. At least 50 bases of flanking intron sequence was included in each amplified exon. RT/PCR products were obtained from liver, lymphoblastoid, or fibroblast samples. Sequencing was performed using radiolabelled dNTPs and cycle sequencing. 12 of the patients were homozygous for both their cDNA and genomic DNA changes. 3 patients were heterozygous in both tissues. 9 patients were homozygous for changes in cDNA which were heterozygous in genomic DNA (Hom/Het). We determined changes in these Hom/Het patients from genomic DNA that were not detected in mRNA. We found 2 premature stop codons from single base substitutions, 3 out of frame deletion/insertion changes resulting in premature stop codons, 1 5' UTR change, 1 mid-exon missense mutation, and 2 intron/exon boundary changes near the 3' end of the intron. These findings demonstrate that changes observed in genomic DNA may often have their effect on the transcript rather than the translation process. These findings also suggest robust cellular mechanisms for degrading flawed RNA. This study suggests that whenever practicable mRNA should be studied alongside genomic DNA to better understand the mechanisms of proposed mutations.

A robust multiplex minisequencing assay for rapid screening of common *HBB* and *HBA2* thalassemia mutations.

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The thalassemias are the most common monogenic disorders in the world, and are endemic to many regions in Southeast Asia. We previously developed a rapid single-tube multiplex PCR test to detect the most common deletional determinants of α -thalassemia. We have now developed a complementary assay to enable rapid screening of the most common thalassemia point mutations. The *HBB* and *HBA2* genes were amplified separately or multiplexed together. Excess PCR primers and unincorporated dNTPs were enzymatically degraded. Multiplex minisequencing was then performed on the purified PCR products, using mutation-specific primers containing non-specific 5' tails of differing lengths and the SNaPshot™ reaction mix containing DNA polymerase and ddNTPs, each labeled with a different fluorophore. Minisequencing primers were designed to anneal next to each mutation site, and comprised a total of 7 common *HBA2* mutations (codon0D1bp, Constant Spring, Paksé, Quong Sze, Suan Dok, codon30D3bp, codon59) and 15 common *HBB* mutations (panel I: codon41/42, IVSIIInt654, IVSI nt5, codon17, -28, -29, codon71/72, codon26; panel II: IVSInt1, codon19, codon0, codon43, codon27/28, codon8/9 and codon35). Minisequencing products were separated and detected by capillary electrophoresis on an ABI PRISM™3100, and analyzed using GeneScan™ and Genotyper™3.7 software. Homozygous wild-type or mutant DNA samples displayed only a single colored peak for each mutation site on the electropherogram, reflecting incorporation of either normal or mutant fluorescent ddNTPs. Samples heterozygous for a specific mutation displayed two different-colored peaks for that mutation site, reflecting incorporation of two different fluorescent ddNTPs by the primer. A double blind analysis of 96 thalassemia patient DNA samples of known genotype was performed, of which 87 samples yielded amplified product and all samples were correctly genotyped. We have thus developed a robust, rapid and reliable minisequencing assay to detect the most common non-deletional Southeast Asian thalassemia mutations.

A novel biomarker: somatic transversion mutations of mitochondrial DNA increased in patients with diabetes mellitus. *J. Kamiya¹, Y. Aoki¹, K. Kiyosawa¹, Y. Fukushima².* 1) Dept 2nd Internal Med, Shinshu Univ Sch Medicine, Nagano, Japan; 2) Dept Medical Gen, Shinshu Univ Sch Hospital, Nagano, Japan.

Augmented oxidative stress induced by hyperglycemia possibly contributes to the pathogenesis of diabetic complications. Oxidative stress is known to increase the conversion of deoxyguanosine to 8-oxo-2'-deoxyguanosine (8-oxodG) significantly in the DNA of patients with diabetes mellitus (DM). 8-oxodG can form Watson-Crick pairs with both cytosine (C) and adenine (A), and therefore, it can give rise to guanine (G):C to thymine (T):A or T:A to G:C transversion mutations. Hence, we investigated what kind of somatic mutations actually occurred in the mitochondrial DNA (mtDNA) of diabetic patients, which is thought to be more vulnerable to oxidative damage than nuclear DNA. We studied five diabetic patients (HbA1C = $8.27 \pm 0.60\%$), and five age-matched healthy control subjects. We subcloned and sequenced two parts of mtDNA (control region (nt15996-16401, segment A) and the segment encompassing t-RNA^{Leu(UUR)} (nt3149-3404, segment B)) in the peripheral blood, and identifying somatic mutations from 20 colonies each, while assaying urinary 8-oxodG by the ELISA detection method. The number of total somatic mutations, each of which was not over 5% in heteroplasmy, had no difference between patients with DM and control subjects (19, 16 in DM patients vs 23, 11 in healthy controls, in Segment A, B respectively). However, transversion somatic mutations of the mtDNA significantly increased in patients with DM (5, 6 vs 1, 0; $P < 0.001$, according to two-sided Fisher's exact test), including the three mutations presumably due to 8-oxodG; while transition mutations did not significantly increase. The amount of transversion mutations was not correlated with the content of 8-oxodG in the urine, which tended to increase in diabetic patients, without a significant difference. This is the first report to show somatic transversion point mutations of mtDNA increase in patients with DM, even when not associated with urinary 8-oxodG. Such transversion mutations can become a novel biomarker of mtDNA damage associated with hyperglycemia, and possibly with oxidative stress which is not reflected by urinary 8-oxodG.

Thanatophoric dysplasia type II mice generated using cartilage-specific cre. *T. Lin, S.B. Sandusky, Z. Zhang, C.A. Francomano.* Laboratory of Genetics, National Inst on Aging/NIH, Baltimore, MD.

Fibroblast growth factor receptors (FGFR) are tyrosine kinase receptors, which bind extracellular FGF ligands. FGFR3 is of particular interest since numerous mutations in this gene give rise to human skeletal dysplasias. Specific mutations have already been identified in hypochondroplasia (HCH), thanatophoric dysplasia (TDII), and severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN). Two dysplasias of interest to our laboratory, TDII and SADDAN, are caused by mutations in K650 of human FGFR3 in the second tyrosine kinase domain. To gain a better understanding about their phenotypic differences and to elucidate the underlying molecular bases of these growth disorders, TDII and SADDAN mouse models of FGFR3 were designed with point mutations (K644E and K644M) accurately reflecting the phenotype of the particular skeletal dysplasia. In order to overcome lethal effects caused by the mutation, an in vivo Cre-loxP recombination strategy was used where neo was used to suppress the mutation that the mice were carrying, (FGFR3^{+/neo-K644E})(FGFR3^{+/neo-K644M}) thus generating mice that were phenotypically normal. We have generated our first tissue specific TDII mice by crossing FGFR3^{+/neo-K644E} transgenic mice with cartilage specific COL2A1-Cre mice. The profound phenotype is similar to that of the TDII with K644E expression in all cells. These tissue specific TDII pups had a shorter tail (half of the length of their wild type litter mates) with caudal widening, extremely short, curved forelimbs and hind limbs, and a slightly enlarged head. Upon dissecting the ventral portion of their rib cage, the TDII pups showed features including a much smaller and narrower rib cage than their wild type littermates. Little or no milk was observed in their stomach, indicating decreased viability, while some of their littermates had milk 2 hours after birth. Currently, we are growing the isolated chondrocytes in vitro in alginate beads from these postnatal TDII pups for cellular and molecular characterization.

Modeling Spinal and Bulbar Muscular Atrophy in a Mouse. *C.J. O'Brien, E. Chevalier-Larsen, H. Wang, S. Jenkins, D.E. Merry.* Dept. of Biochemistry & Molecular Pharmacology, Thomas Jefferson University, Philadelphia PA.

Spinal and Bulbar Muscular Atrophy (SBMA) is an adult onset, progressive motor-neuronopathy attributable to the expression of an expanded polyglutamine tract in the N-terminal region of the androgen receptor (AR) protein. Symptoms include bulbar and proximal limb muscular atrophy with fasciculations, and mild androgen insensitivity. Histology reveals neuronal intranuclear inclusions (NII) and the depletion of motor neurons residing in the spinal cord and brainstem. We created a model of SBMA, using transgenic (TG) mice that bear the full-length mutant AR112Q cDNA driven by the prion protein (PrP) promoter. Characterization of the model reveals a gender dependent neurologic disease, suggesting a ligand dependent process. By two months of age, TG males exhibit motor deficits and NII formation. TG females display a mild and delayed phenotype, without notable muscular impairment and few NII. TG AR24Q animals are phenotypically normal and lack NII. Immunohistology (IHC) of brain and spinal cord from TG AR112-34 animals reveals neuronal nuclear staining in brain and spinal cord. Punctate NII are detected in TG males at two months of age with the N-terminal antibodies AR (N-20) and AR318. IHC with antibodies AR441 and AR (C-19) diffusely illuminate the nucleus, but failed to detect these same NII. Protein analysis of AR112 reveals that AR (N-20) positive NII are insoluble in Triton, and are rendered only partially soluble in 4% SDS, suggesting biochemical differences between these NII, and the SDS-insoluble NII seen in previous models using a truncated AR. NII in the TG PrpAR112Q mice resemble those seen in SBMA patients, which are characterized by the absence of C-terminal epitopes. The presence of selected epitopes in NII provides a system with which to investigate the role of AR proteolysis in the onset and progression of SBMA. The gender preference of onset and progression in our model likely results from the same pathogenic mechanism as in SBMA. Ours is an appropriate model for the further examination of ligand dependent mutant AR proteolysis, and the effects of AR ligands on the onset, progression, and phenotype of this neuromuscular disease.

A Murine Model for Hereditary Hemorrhagic Telangiectasia Type 2. *S. Srinivasan¹, T. Dickens¹, L.P. Hale², D.A. Marchuk¹*. 1) Department of Genetics; 2) Department of Pathology, Duke University Medical Center, Durham, NC, 27710.

Hereditary Hemorrhagic Telangiectasia (HHT) is an autosomal dominant disorder characterized by multi-systemic vascular lesions that are prone to hemorrhage. The hallmark vascular lesions, arteriovenous malformations (AVMs), can occur in major organs such as the brain, lung, gastro-intestinal tract, liver, and skin (especially muco-cutaneous tissues). Penetrance is age-dependent and nearly complete only by the 4th decade. HHT type 2 is caused by loss of function mutations in activin-receptor-like kinase-1 (Alk-1). Disruption of the murine Alk-1 gene results in embryonic lethality in the homozygotes due to severe vascular abnormalities (Oh et al., Proc Natl Acad Sci USA 2000, 97:2626-31). We have investigated whether mice heterozygous for Alk-1 deletion will serve as an appropriate animal model for HHT type 2. Alk-1 heterozygotes were maintained for a period of up to 15 months in order to permit the development and progression of an HHT-like phenotype. Five Alk-1 heterozygotes died prematurely, of which, two exhibited vascular abnormalities and severe hemorrhage in a variety of organs. Hemorrhage was evident in the nailbeds, the spleen, and the liver. In addition, dilated and entangled vessels were observed in the paws and on the tongue. Mice were sacrificed at various ages (6-15 months) and examined for the presence of vascular anomalies. Vascular lesions and hemorrhage were identified in 1 of 8 heterozygotes. The anatomical locations of these lesions resemble those of the human disorder. These results suggest that despite the possibility of reduced penetrance, Alk-1 heterozygous mice are an appropriate animal model to investigate the pathogenesis of HHT type 2.

Inducible mice model of Spinocerebellar Ataxia Type 3. *Th. Schmidt¹, J. Boyl¹, C. Holzmann², S. Ibrahim³, U. Grasshoff¹, I. Schmitt⁴, F. Zimmermann⁵, S. Prusiner⁶, O. Riess¹.* 1) Department of Medical Genetics, University of Tübingen, Germany; 2) Department of Medical Genetics, University of Rostock, Germany; 3) Department of Immunology, University of Rostock, Germany; 4) Department of Neurology, University of Bonn, Germany; 5) ZMBH, University of Heidelberg, Germany; 6) Department of Neurology, University of California, San Francisco, CA.

Spinocerebellar Ataxia Type 3 (SCA3) or Machado-Joseph-Disease (MJD) is an autosomal dominantly inherited neurodegenerative disorder caused by the expansion of a CAG stretch in the *MJD1* gene encoding a polyglutamine repeat in the respective ataxin-3-protein. In order to study the course of the disease we generated an inducible transgenic mouse model using the Tet-Off-System developed by Dr. Bujard (Heidelberg). This system is based on two constructs: The promoter construct controls the expression of the so called tTA (Tetracycline transactivator) gene product. The binding of this protein to a Tetracycline responsive element (TRE) in the responder construct induces the transcription of the gene of interest. The expression can be blocked by the addition of Tetracycline which allosterically inhibits the tTA protein. For the ataxin-3-responder mouse lines two different full length constructs containing 15 repeats (control lines) and an expanded repeat with 77 glutamines (disease model) were used.

So far three founders for the control line and six founders for the disease model were generated and stable mouse lines established. In order to verify the function of these mouse lines we crossbred the ataxin-3 responder lines with a well-characterized prion protein promoter mouse line (kindly provided by Dr. S. Prusiner). Double transgenic mice of two disease model mouse lines express the ataxin-3 protein with an expanded polyglutamine repeat. Studies are ongoing to characterize these mouse lines and to define their suitability as a model of SCA 3. These models will allow us to turn off or on ataxin-3 expression at different developmental stages and will demonstrate whether or not the disease phenotype will be reversible.

Functional analysis of lamin A mutations underlying Emery-Dreifuss muscular dystrophy. *C.A. Brown¹, I. Motsch², C. Hartmann³, J. Zhang³, J.A. Ellis².* 1) Dept Lab Med & Pathology, Univ Minnesota Med Sch, Minneapolis, MN; 2) Kings College, London, UK; 3) Carolinas Medical Center, Charlotte, NC.

Emery-Dreifuss muscular dystrophy (EDMD) is characterized by slowly progressive muscle wasting and weakness, early contractures of the elbows, Achilles tendons and spine, and cardiomyopathy associated with cardiac conduction defects. Mutations in the LMNA gene, encoding lamins A and C, have been reported in patients with autosomal dominant, autosomal recessive, and sporadic forms of EDMD. We have examined the effect of nine mutations found in EDMD patients on subcellular localization of mature lamin A, and/or of endogenous nuclear antigens such as emerin, lamin C and lamin B. Transient expression of pEGFP constructs was performed in the mouse myoblast cell line C2C12 and the monkey fibroblast cell line COS-7. Confocal microscopy was used to determine the location of the synthesized EGFP-lamin A fusion protein in these cells. Constructs carrying the lamin A mutations R453W, R527P, L530P were localized normally to the nuclear envelope and neither endogenous emerin or lamin C localization were affected. The G602S and 190+R mutations were also localized normally to the nuclear envelope. Mutations E358K and T150P produced a mislocalization of exogenous lamin A and a rearrangement in endogenous emerin and lamin C localization. The E358K mutant produced a diffuse nucleoplasmic staining, and caused endogenous lamin C to take up a similar appearance. Endogenous emerin in this instance was less distinct in the nuclear envelope. The T150P mutant lost nuclear lamina and nucleoplasmic punctate staining, and became completely punctate and polarized to one side of the nuclear envelope. Endogenous lamin C took up a more punctate appearance within the nucleoplasm. The E361K and R133P constructs demonstrated intranuclear foci, consistent with the inability of the altered lamin A to incorporate into the nuclear lamina. These results indicate the variable effect of pathogenic forms of lamin A at the cellular level.

A unique structure-function analysis of a mutation in the androgen receptor gene that causes complete androgen insensitivity. *B. Gottlieb*^{1,5}, *J.H. Wu*^{3,5}, *T.C. Scanlon*^{1,2}, *S. Ghali*^{1,2}, *L.K. Beitel*¹, *M.A. Trifiro*^{1,4}. 1) Dept Cell Genetics, Lady Davis Inst Medical Res, Montreal, PQ, Canada; 2) Department of Human Genetics, McGill University, Montreal; 3) Department of Oncology, McGill University, Montreal; 4) Department of Medicine, McGill University, Montreal; 5) Center for Translational Research in Cancer, McGill University, Montreal.

An androgen receptor (AR) gene mutation (R774C) in the ligand binding domain (LBD) of the AR results in complete androgen insensitivity due to the receptor's inability to bind ligand. This is typical of many AR LBD mutations that are either not in the putative ligand binding pocket (LBP), or do not result in gross structural LBD alterations. To understand the possible mechanism of action of such mutations, the structure/function relationship of R774C has been analyzed by performing a molecular dynamic simulation of 1.4 ns using the Generalized Born model 2 as implemented in the Amber 7 package. The average structure of the mutant showed that the mutation had local structure distortion in the LBD. Part of AR helix 5 rewinds into a loop, which in turn causes movement of loops 759-772 and 682-695. The movement of loop 682-695 in particular, results in a change in shape of the LBP, and movement of the bound ligand within the pocket. This result is significant, as this is the first case of modeling showing an effect on ligand binding, of a mutation not within the LBP, that has a subtle effect on LBD structure. To confirm the validity of this observation, photoaffinity cross-linking experiments are underway. The normal and mutant AR LBDs were cloned into a GST bacterial expression system. Following protein expression, the cell cultures were incubated with the synthetic ligand [³H] R1881, the cells exposed to UV radiation and the GST-fusion proteins purified. The cross-linked fusion proteins were then cleaved with trypsin and Asp-N, run on a SDS-PAGE gel, which was exposed to film. Any resulting differences in R1881 cross-linking between normal and mutant receptors would validate the modeling technique and allow for analysis of many more non-LBP LBD mutations, not just in AR, but also in other members of the super-family of steroid receptors.

Hearing loss: frequency and functional studies of the most common CX26 alleles. *P. Gasparini*¹, *V. Veronesi*², *M. Bicego*², *S. Melchionda*³, *L. Zelante*³, *E. Di Iorio*⁴, *R. Bruzzone*⁵, *P. D'Andrea*². 1) Medical Genetics, SUN-TIGEM, Napoli, Italy; 2) Dip Biochimica, Biofisica e Chimica delle Macromolecole, Univ Trieste, Italy; 3) Serv Genet Med, IRCCS-CSS, San Giovanni R, Italy; 4) TIGEM (Telethon Institute of Genetics and Medicine), Naples, Italy; 5) Dpt Neurosci, Institut Pasteur, Paris, France.

Despite the large genetic heterogeneity in hearing loss, mutations in GJB2 (connexin 26) account for the majority of recessive and some of dominant cases of deafness. After a large screening of deaf patients we were able to define the frequency of GJB2 alleles in our Italian population. Six of them are missense mutations. In these cases functional studies have been carried out using biochemical and cell biological approaches. Briefly, the coding region of either wild-type (wt) or mutated Cx26 was subcloned into pIRES-EGFP, which permits both the gene of interest and the enhanced form of *Aequorea victoria* GFP gene to be translated from a single bis-cistronic mRNA, allowing an efficient selection of transfected cells for functional studies. To study the cellular localization of mutated Cx26 proteins, HeLa cells were transiently transfected with the cDNA for wtCx26 and relative mutations. To examine to which extent Cx26 mutations affected the level of protein expression, Western blot experiments were performed on total lysates of transiently transfected cells. Finally, to examine whether the mutant connexins could form functional gap junctions, the intercellular transfer of Lucifer yellow was analysed in cells transiently expressing the mutated connexins. As expected, absence of the protein was detected with the most common 35delG mutation. Regarding missense mutations, our data demonstrate that S19T, G12V, M34T, L90P, R184P and R127H lead to abnormal protein in terms of expression, localization and/or functionality. Moreover, M34T acts as a dominant inhibitor of wild type Cx26 channel activity when co-expressed in communication-deficient HeLa cells. These data are an useful step towards a complete understanding of the role of GJB2 in causing hearing loss.

Molecular basis of Peters' anomaly in a cohort of Saudi Arabian patients: expanding the phenotypic spectrum of *CYP1B1* mutations. *B.A. Bejjani*¹, *S.M. Curry*¹, *R.A. Lewis*¹, *A. Al-Rajhi*², *D. Edward*³. 1) Dept Molecular/Human Genetics, Baylor Col Medicine, Houston, TX; 2) King Khaled Eye Specialist Hospital, Riyadh, Saudi Arabia; 3) University of Illinois in Chicago, Chicago, IL.

Peters' anomaly (PA) is a developmental defect of the anterior segment that may occur as an isolated event or that may be associated with ocular and systemic defects. It is phenotypically and genotypically heterogeneous. We sought to determine the role of *CYP1B1* mutations in a cohort of 10 Saudi Arabian families segregating PA in 11 subjects. Experienced ophthalmologists diagnosed all subjects. The diagnosis was confirmed by pathological examination of corneal buttons in 5 subjects. Three subjects from 2 large families had siblings and cousins affected with Primary Congenital Glaucoma (PCG). Purified DNA was prepared from peripheral blood samples and *CYP1B1*-coding exons were amplified and sequenced. The molecular bases of PA and PCG were determined in 6 individuals from 5 families: A) a single missense mutation in *CYP1B1* (G61E) was identified in 5 individuals with PA in 4 families. This is a common in PCG mutation described previously in PCG patients from many ethnic backgrounds; B) a homozygous 10 base-pair deletion that yields a null allele by conceptual translation was detected in a patient with PA and in his siblings with PCG. Importantly, These 2 large families include 3 individuals (2 in one family, 1 in another) with homozygous *CYP1B1* mutations and no ocular anomalies (nonpenetrant). Sequence analysis of coding exons of genes known to be associated with PA (*PAX6*, *FOXC1*, and *FOXE3*) revealed no mutations in any subjects tested. Thus, mutations in *CYP1B1* may be a major cause for PA in this population and also, PA and PCG may share common molecular pathways. We also suggest that PA and PCG may represent variable severity in the same spectrum of anterior segment dysgeneses. The occurrence of PA, PCG, and unaffected individuals with identical homozygous *CYP1B1* mutations in the same sibship could suggest the presence of modifiers that modulate the clinical severity of the phenotypic expression of the same *CYP1B1* mutation(s).

Genetic Homogeneity of G6PD Deficiency in Bahrain. *N.M. Jassim, S.S Al Arrayed.* Genetic department, Salmaniya Medical Complex, Manama, Bahrain.

Glucose 6-Phosphate Dehydrogenase is an enzyme that is essential for protection of red blood cells from oxidation. Deficiency of G6PD activity is presumed to be the most common enzymopathy in man, with over 100 mutant alleles causing the phenotypic deficiency. Estimation of G6PD deficiency in Bahrain showed high prevalence of deficient subjects rating up to 26% of the whole population. Previous PCR-based DGGE, and RFLP molecular investigations, we found that the main causal defect of G6PD deficiency in Bahrain is the common variant G6PD Mediterranean (nt 563 C-T). Here, we studied 60 unrelated phenotypically deficient subjects (48 males and 12 females). We found that 70 out of 72 X-chromosomes (97%) are bearing the G6PD Med variant. Further analysis of the silent polymorphism at nucleotide position 1311 of this gene revealed quite homogeneity for this mutation in Bahrain. Out of 46 subjects (34 males and 12 females) bearing the G6PD Med mutation. 56 X-chromosomes (97%) showed thymine (T) at nt 1311, while it is cytosine (C) in two X-chromosomes (3%). In contrast, we studied 87 X-chromosomes for this silent polymorphism from subjects of normal phenotype and genotype for this enzyme. We found 52 X-chromosomes (60%) having "C" at nt 1311 and 35 X-chromosomes (40%) having "T". In conclusion these findings revealed high genetic homogeneity for G6PD deficiency in Bahraini people. Furthermore, the presence of thymine at nt 1311 for the vast majority of mutant chromosomes might suggest a possible unicentric source of this mutation in Bahrain.

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Carrier frequency of DF508 mutation in the CFTR gene in Indian population. *V. Kapoor, M. Kabra, S.S. Shastri, R. Vijaya, S. Arora, B. Prahlad, S.K. Kabra, A.K. Deorari, V.K. Paul, P.S.N Menon.* Department of Pediatrics, All India Institute of Medical Sciences, New Delhi, Delhi, India.

Cystic Fibrosis (CF) is a chronic, progressive, autosomal recessive disease of the exocrine glands involving multiple organs resulting in diverse phenotype. Mutations in Cystic Fibrosis Transmembrane conductance Regulator (CFTR) gene cause altered function of chloride channels on the apical membrane of the epithelial cells. More than 1000 mutations in the CFTR gene have been reported to the Cystic Fibrosis Genetic Analysis Consortium, of which DF508 mutation is the commonest accounting for 70-80% of all mutations causing CF in the Western population. CF is considered to be rare in Asians (i.e. 1/10,000) based in Goodchild et al study in the migratory Asian population of UK. The prevalence of DF508 mutation in Asians is 19-44% based on a few case reports. There are no population based studies on the prevalence of CF in India. This study was undertaken to estimate the carrier frequency of DF508 mutation in the Indian population. Nine-hundred and fifty five samples were collected in EDTA vacutainers. DNA was extracted by phenol-chloroform method and tested for DF508 mutation by PCR according to the protocol of Chris Mathews et al. The PCR products were analysed in 8% polyacrylamide gel followed by silver staining. On testing 955 cord blood samples, 4 samples were heterozygous for DF508 mutation. The place of origin of the parents in these four cases was Uttar Pradesh, Jammu, Karnataka and Pakistan respectively. Though the majority of the parents were from North India, mainly from Delhi and the adjoining States we had one case from South India. Based on this data, the carrier frequency of DF508 mutation was 1/238 (i.e. 0.42%). With the available data, using the Hardy-Weinberg principle the frequency of CF disease caused by DF508 homozygosity was calculated to be 1/2,28,006. The estimated prevalence of CF in Indian population ranges from 1/43,321 to 1/1,00,443. Thus CF does occur in the Indian population though the frequency is less as compared to the Caucasians. Considering the genetic diversity of the Indian population, further studies are required on a larger sample size.

cDNA microarray analysis of single patient with Duchenne muscular dystrophy. *S. Noguchi*^{1,2}, *T. Tsukahara*¹, *R. Kurokawa*^{1,2}, *M. Fujita*^{1,2}, *Y.K. Hayashi*¹, *Y. Goto*¹, *I. Nonaka*¹, *A. Tujimoto*³, *I. Nishino*^{1,2}. 1) Natl Inst Neuroscience, NCNP, Kodaira, Tokyo, Japan; 2) CREST, JST, Kawaguchi, Saitama, Japan; 3) DNA Chip research Inc, Yokohama, Kanagawa, Japan.

We newly developed a microarray containing 4224 human cDNA clones representing 3500 genes expressed in skeletal muscles. Our cDNA microarray system provides both high sensitivity and reproducibility with only 1 microgram of total RNA which first allowed us to study gene expression in a single patient specimen. We analyzed six muscle specimens from patients with Duchenne muscular dystrophy (DMD) both to deduce common pathognomonic features in DMD and to highlight difference among patients at the molecular level. The genes associated with myofiber degeneration, cell infiltration, inflammation, and muscle development, were significantly upregulated, while those related to muscle homeostatic function were downregulated. Among the genes regulated differently among the patients, we focused on the expression of the genes encoding HLA-related proteins, and the myosin light chain and troponin T isoforms as parameters for necrotic and regenerating process. The expression pattern of these genes was in accordance with the severity of dystrophic changes on muscle pathological examination. Our new cDNA microarray provides a molecular basis of muscle pathology.

CARTILAGE HAIR HYPOPLASIA (CHH) AND THE RNASE MRP (RMRP) GENE. *P. Hermanns¹, A. Bertuch¹, E. Munivez¹, A. Mian¹, V. Lundblad¹, J. Leroy¹, B. Zabel², B. Lee¹.* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Children's Hospital of the University of Mainz, Mainz, Germany.

Cartilage Hair Hypoplasia (CHH) is an autosomal recessive disorder characterized by, short stature, blond sparse hair, defective cellular immunity and predisposition to several cancers. CHH patients have mutations in the RNase MRP (RMRP) gene and one Finnish allele accounts for a majority of mutations. RMRP is the RNA component of a ribonucleoprotein which functions as an endonuclease and is thought to be involved in processing of the pre-rRNA in the nucleolus, cleavage of mitochondrial RNA, and priming of mitochondrial DNA replication. Its function in vertebrates and the pathogenesis of CHH is unknown and it is the first nuclear encoded RNA to be linked to a Mendelian genetic disease. In a cohort of 14 patients we found mutations in both alleles in 12 patients. Two patients had no mutations in the RMRP gene. Ten mutations have not been previously described. The mutations include insertions in the promoter region, missense substitutions of evolutionarily conserved as well as divergent bases. We have not found high numbers of polymorphism in outbred populations. The transcribed part of the RMRP gene is highly conserved among different species; however, the only mutation which is conserved between human and yeast is the main Finnish mutation (A70G). This mutation has been reported NOT to reduce the RMRP levels or to alter protein-RMRP level and protein-RMRP binding. The RMRP protein complex still forms (Ridanp et al. Cell 2001). To further understand the potential function of RMRP in cellular metabolism, we introduced the A70G mutation into the yeast ortholog of RMRP (NME1). The NME1 null allele causes lethality in yeast. In contrast, the A70G allele did not exhibit differences regarding growth rate, nutritional requirement, or chromosomal stability at different temperatures. Current studies are focused on generational differences for these parameters.

The Pyrin Pathway of Inflammation: Arthritis-Associated Mutations in PSTPIP1 Cause Hyperphosphorylation and Increased Binding to Pyrin. *N.G. Shoham¹, E. Mansfield¹, M. Centola¹, G. Wood¹, C.A. Wise², D.L. Kastner¹.* 1) Genetics and Genomics, NIAMS, NIH, Bethesda, MD; 2) Molecular Genetics and Cellular Biochemistry, center for musculoskeletal research, Scottish Rite Hospital, Dallas, Texas.

Pyrin, the FMF protein, is the prototype of a family of proteins that share an N-terminal death domain fold motif (the pyrin domain) and have been implicated in apoptosis and inflammation. To identify other associated proteins, we used pyrin as the bait in a yeast two-hybrid assay. One of the pyrin-interactors was proline serine threonine phosphatase-interacting protein (PSTPIP1), a tyrosine-phosphorylated protein previously shown to interact with PTP-PEST, c-Abl, CD2, and the Wiskott-Aldrich syndrome protein (WASP). PSTPIP1 and pyrin were found at high levels in monocytes, and, when cotransfected in HeLa cells, they colocalized by fluorescence microscopy and could be coimmunoprecipitated. Deletion mutants demonstrated that the B-box/coiled-coil segment of pyrin is necessary and sufficient for this interaction. In contrast, we found that the SH3 and coiled-coil domains of PSTPIP1 are both necessary, but neither is sufficient, for pyrin-binding. The Y344F point mutation, which blocks the tyrosine-phosphorylation of PSTPIP1, was associated with a marked reduction in pyrin-binding in pervanadate-treated HeLa cells. Further, we found that the A230T and E250Q PSTPIP1 mutations, which were recently shown to cause the dominantly inherited syndrome of pyogenic arthritis, pyoderma gangrenosum, and acne (PAPA), caused increased pyrin-binding as assayed by immunoprecipitation. Relative to wild-type, these PSTPIP1 mutants were hyperphosphorylated when coexpressed with c-Abl kinase. Taken together, these data suggest that PAPA-associated mutations increase PSTPIP1 phosphorylation and consequently pyrin-binding, thus providing a possible mechanism for the PAPA syndrome.

Mutations in the small GTPase late endosomal protein RAB7 are associated with Charcot-Marie-Tooth type 2B neuropathy. V. Timmerman¹, K. Verhoeven¹, K. Coen¹, N. Verpoorten¹, M. Auer-Grumbach³, J.M. Kwon⁴, D. FitzPatrick⁵, E. De Vriendt¹, A. Jacobs¹, V. Van Gerwen¹, K. Wagner³, H.P. Hartung⁶, P. De Jonghe^{1,2}. 1) Molecular Genetics Department, Flanders Interuniversity Institute for Biotechnology, Born Bunge Foundation, University of Antwerp, Antwerpen, Belgium; 2) Division of Neurology, University Hospital Antwerpen, Antwerpen, Belgium; 3) Institute of Medical Biology and Human Genetics, Karl-Franzens University Graz, Austria; 4) Department of Neurology, Washington University School of Medicine, St. Louis, MO, USA; 5) South East Scotland Clinical Genetic Service, Western General Hospitals, Edinburgh, Scotland; 6) Department of Neurology, Heinrich-Heine University, Dusseldorf, Germany.

Ultero-mutilating neuropathies are characterised by prominent sensory loss, often complicated by severe infections, arthropathy and amputations. So far, two loci and one gene have been reported for autosomal dominant ultero-mutilating neuropathies. However, molecular genetic studies have demonstrated that a third locus has to exist. Hereditary sensory neuropathy type I (HSN I) maps to 9q22.1-q22.3 and is caused by mutations in the SPTLC1 gene. The locus for hereditary motor and sensory neuropathy type IIB (HMSN IIB) or Charcot-Marie-Tooth type 2B (CMT2B) maps to 3q13-q22. CMT2B is clinically characterised by marked distal muscle weakness and wasting, a high frequency of foot ulcers, infections and amputations of the toes due to recurrent infections. We found two missense mutations (L129F and V162M) in the small GTP-ase late endosomal protein RAB7 associated with the CMT2B phenotype in three families. The alignment of RAB7 orthologs shows that both missense mutations target highly conserved amino acid residues. The V162M mutation affects a valine that is conserved among all species. The L129F mutation is localised next to a conserved GTP-binding domain. RAB7 is known to be ubiquitously expressed, and we found expression in mouse E13 sensory and motor neurons. Why mutations in an universally expressed protein as RAB7 lead to an axonal pathology in CMT2B is currently unknown.

Disease-specific changes in facioscapulohumeral muscular dystrophy by expression profiling. *Y.-W. Chen¹, P. Masny², K.M. Flanigan³, J. Martin², H. Qian¹, T. Mozaffar⁴, S.T. Winokur².* 1) Center for Genetic Medicine, Children's National Medical Center, and George Washington University, Washington, DC; 2) Department of Biological Chemistry, University of California, Irvine, CA; 3) Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, UT; 4) Department of Neurology, University of California, Irvine, CA.

Facioscapulohumeral muscular dystrophy (FSHD) is an inherited muscle disorder resulting from deletions of the 3.3 kb D4Z4 repeat units on 4q35. Although a position effect hypothesis is proposed to be the cause of the FSHD, the primary and downstream gene changes of the disease are not clear. To identify FSHD-specific expression changes, we report expression profiling of FSHD muscles using Affymetrix HGU133 set representing 42,000 transcripts. By comparing profiles of 5 FSHD to 4 normal controls, we identified 1014 genes and ESTs significantly ($p < 0.05$) misregulated in the FSHD muscles. After subtracting genes differentially expressed in the Duchenne muscular dystrophy and dysferlin deficiency from that of the FSHD, we identified 668 FSHD-specific changes. The largest functional group of the 374 down-regulated genes was involved in protein synthesis and processing including methionyl aminopeptidase 2, amino acid transporter 2, and ribosomal proteins. As FSHD often has an inflammatory component, it is interesting to note that several immune response genes (eg. CD14, immunoglobulin kappa constant, and complement component 3a receptor 1) were up-regulated more than two fold. In addition, a custom glass slide array consisting of ~200 genes and ESTs from 4q35 and 10q26 was constructed, and 35 genes differentially expressed (31 up- and 4 down-regulated) more than 2 fold were identified in the FSHD samples. Among them, 15 genes and 9 genes were located at the 4q35 and 10q26 regions, respectively. Our results suggest that the 4q35 deletion in FSHD causes primary up-regulation of a subset of genes at both the 4q35 and homologous 10q26 loci, with subsequent downstream dysregulation of a series of disease-specific transcripts. The late onset and asymmetric distribution of muscle involvement may be mediated by metabolic and immune downstream changes.

Microarray analysis of Emery-Dreifuss muscular dystrophy. *Y.K. Hayashi^{1,2}, S. Noguchi^{1,2}, M. Fujita^{1,2}, R. Kurokawa^{1,2}, K. Goto^{1,2}, T. Tsukahara^{1,2}, I. Nishino^{1,2}.* 1) Neuromuscular Research, National Institute of Neuroscience, NCNP, Tokyo, Japan; 2) CREST, Japan Science and Technology Corporation, Saitama, Japan.

Emery-Dreifuss muscular dystrophy (EDMD) is slowly progressive muscular disorder characterized as scapulothoracic-peroneal muscle weakness and atrophy, early joint contractures, and cardiomyopathy with conduction block. X-linked recessive form (X-EDMD) is caused by mutations in the emerin gene on Xq28, and autosomal dominant form is caused by mutations in the lamin A/C gene on 1q21. Mutations in the lamin A/C gene also cause clinically distinct disorders including limb-girdle muscular dystrophy, familial dilated cardiomyopathy, familial partial lipodystrophy, and hereditary axonal neuropathy. Both emerin and lamin A/C are nuclear envelope proteins, however their detailed functions are not known yet. In this study, we characterized the expression pattern of over 5,000 genes in human skeletal muscle using our original cDNA microarray. Our microarray was designed to select genes expressed in human skeletal muscle. Further, this microarray can analyze the gene expression profiles of each patient since only small amounts of total RNA are required. We examined biopsied skeletal muscle specimens from two X-EDMD and one AD-EDMD patients. Human skeletal muscle total RNA (ORIGENE) was used as the reference and the gene expression level was calculated as relative ratio to the reference. Gene clusters were also generated and the gene expression pattern differences between X- and AD-EDMD were compared. The down-regulated 200 genes were involved in 19 energy metabolism, 18 sarcomeric, 20 transcription/translation and 10 DNA binding proteins. The genes of titin and nebulin were down-regulated that were observed in other muscular dystrophies. The up-regulated 200 genes included 31 energy metabolism, 28 sarcomeric and 15 signal transduction. Actin genes, tropomyosin 1, and muscle specific enolase were increased. Immune response genes were not significantly increased which suggested to reflect the pathological changes with no active muscle fiber necrosis. We are now performing detailed analysis of the data.

Malignant Hyperthermia Susceptibility: diagnosis in the South African context. *F.F. Maree^{1,2}, B. Wedge¹, D. Prosser¹, G.S. Gericke³, A. Olckers^{1,2}.* 1) Centre for Genome Research, Potchefstroom University, Pretoria, South Africa; 2) DNAbiotech, Pretoria, South Africa; 3) Medical Research Council, South Africa.

Malignant Hyperthermia (MH) is an autosomal dominant genetically and clinically heterogeneous pharmacogenetic disorder. Traditionally the diagnosis of MH was based on the highly invasive in vitro contracture test (IVCT). This type of diagnostic testing is expensive and cumbersome to provide to the South African population. The promise of an unequivocal molecular test, requiring a mere blood sample, or blood spot, is attractive and logistically feasible in our developing country. The high level of genetic heterogeneity in MHS has prompted active debate on the suitability of a molecular strategy as the sole diagnostic approach for MH. In South Africa the two IVCT diagnostic centres which were operational in the past have closed down, leaving us with no alternative but to embrace a molecular diagnostic strategy. Samples referred to us, from both private and public clinics, are subjected to the screening of the 15 causative mutations within the RYR1 gene, as outlined by the European MH Group. Failing the detection of any of these mutations, samples are subjected to a mutation screening strategy in order to identify novel mutations associated with MH in the South African population. To date, we have received 208 samples, of which 11 (all from the same family SA105) has been identified to harbour the Arg614Cys mutation in the RYR1 gene on chromosome 19q. Seven MH loci have been reported in the human genome. Our initial approach was to classify families, after haplotype analysis, as displaying linkage to either of these loci. Via this approach one family, SA102, has been identified to link to a novel locus on chromosome 2q, as reported earlier. Here, our in silico approach to elucidate the genetics of MH holds promise but, to date, no candidate gene for MH has been identified at this locus. The strategy to screen for MH on the molecular level has also been adopted by other countries in Europe and North America. However, the genetic heterogeneity of MH argues in favour of high throughput facilities where multiple mutations can be screened simultaneously.

Molecular exploration of the Dysferlin gene in LGMD2B and Miyoshi myopathy in a large cohort of patients. K.

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Limb Girdle Muscular Dystrophies (LGMD) are clinically heterogenous disorders with different modes of inheritance. Among the autosomal recessive forms of LGMD, LGMD2B involving proximal muscles at the onset, and distal Miyoshi myopathy are due to mutations in the same gene dysferlin. This gene encompasses 55 exons spanning over 150 kb of genomic DNA. It encodes a membrane associated protein of unknown function containing 2088 aminoacids. Our purpose was to perform a mutational analysis in a serie of patients presenting with a proximal phenotype (LGMD2B) or a distal myopathy of Miyoshi type. All patients had a deficiency in protein dysferlin showed in immunohistochemistry or Western-blot analysis. Mutational screening was done in the 55 exons followed by sequencing for exons with abnormal migration profile. This analysis revealed 8 new homozygous or compound heterozygous mutations in LGMD and Miyoshi patients. Phenotype-genotype correlations have been established and revealed that variable phenotypes may be associated to a same mutation in unrelated patients as well as in a same family. Among these mutations, a homozygous (1765-1766 insA) in exon 15 leading to a premature stop codon (R589fsX608) was found in a patient with atypical Miyoshi myopathy. This mutation is predicted to result in a complete loss of function of the dysferlin protein and will be particularly discussed. Also, a splicing mutation has been found in 3 different French patients, suggesting a mutation "hotspot" in this population. This study shows that a clinical approach followed by a protein analysis are absolutely necessary before performing mutational screening in the general strategy of diagnosis of the LGMD.

Fukutin is required for maintenance of muscle integrity, cortical histiogenesis, and normal eye development. S.

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Fukuyama-type congenital muscular dystrophy (FCMD) is characterized by congenital muscular dystrophy associated with brain malformation (polymicrogyria) due to a defect during neuronal migration. We previously identified the gene responsible for FCMD, which encodes the fukutin protein. Most FCMD patients carry an ancestral mutation, which arose as a consequence of the integration of a 3-kb retrotransposon element into the 3' untranslated region of the fukutin gene. No FCMD patients have been identified with non-founder (point) mutations on both alleles, suggesting that such patients are embryonic-lethal and fukutin is essential for normal development. Here we report that chimeric mice generated using ES cells targeted for both fukutin alleles develop severe muscular dystrophy, with the selective deficiency of alpha-dystroglycan and its laminin-binding activity. They showed laminar disorganization of the cortical structures in the brain with impaired laminin assembly, focal interhemispheric fusion and hippocampal and cerebellar dysgenesis; and anomaly of the lens, loss of laminar structure in the retina, and retinal detachment. These results indicate that fukutin is necessary for the maintenance of muscle integrity, anchorage by radial cells, neuronal intercellular adhesion, and normal ocular development.

Comparative cross-species profiling of human DMD and mouse models for muscular dystrophy helps identify downstream pathways of disease pathogenesis. *L. Pasquali¹, M. Bakay¹, F.W. Booth¹, Y. Chen², K. Gorni³, B. Tseng¹, P. Zhao¹, E.P. Hoffman¹.* 1) Research Center for Genetic Medicine, Children's National Medical Center, Washington, DC; 2) Department of Veterinary Biomedical Sciences, University of Missouri at Columbia, Columbia, MO; 3) Department of Neurology, University of California, San Francisco, CA.

DMD patients lack dystrophin, and they present chronic degeneration/regeneration of the muscle, with progressive muscle wasting and early death. The *mdx* mouse, despite the complete loss of dystrophin, after initial necrosis, at 3-4 weeks of age, shows successful muscle regeneration, muscle hypertrophy and increased strength. Our goal was to identify compensatory mechanisms induced in the mouse but not human, by comparing the genome-wide expression profiles. The hypothesis is that differentially expressed genes in DMD and *mdx* mouse may confer protection to the murine dystrophin deficient model. We used as a starting point a "late muscle regeneration cluster" generated from a 27 time-point murine regeneration series. We then compared the expression of these genes in dystrophin-deficient *mdx* and experimental muscle regeneration (U74 GeneChip series) and human DMD muscle (U95 GeneChip series and custom Affymetrix MuscleChip). We generated a list of genes that were upregulated in both mouse models, but not upregulated in DMD. We then produced antibodies against each of the gene products to confirm the expression profiling and identify cell types expressing the differentially regulated proteins. These genes, that are inhibitors of negative regulators of skeletal muscle mass, specific cell division and differentiation genes, and specific connective tissue modulatory genes, are pharmacological targets for modulation of the progressive pathology of the human disease.

Identification of a novel frame shift mutation in RSK2 gene from a Coffin-Lowry syndrome family. *T.-J. Chen¹, GL. Wilson¹, Y. Wang¹, P. Maertens², W. Wertelecki¹, J. Martinez¹*. 1) Dept Medical Genetics, Univ South Alabama, Mobile, AL; 2) Dept Neurology, Univ South Alabama, Mobile, AL.

Coffin-Lowry syndrome (CLS) is a X-linked mental retardation syndrome caused by defects on the RSK2 gene. We have previously reported a CLS family with four patients in two generations (AJHG, 61(4): A106, 1997). The patients in this family, a mother and her 3 children (a male and two females), have severe mental retardation with typical CLS phenotype. In addition, brain MRI studies on these patients revealed abnormalities in the deep subcortical white matter, thinning of the corpus callosum, hypoplastic cerebellar vermis, and asymmetry of the lateral ventricles. The severity of the MRI findings correlated with the severity of mental retardation within these patients. Recently, we performed extensive mutation screening on the whole RSK2 gene on this family. Twenty-two exons including the intron/exon junctions have been amplified by PCR and subsequently, sequenced on both strands using ABI 310 automatic sequencer. A novel mutation of two nucleotide insertion (298 ins TG) has been identified on the patient's RSK2 gene. The insertion creates a stop codon on codon position 100, and the truncated RSK2 protein only has 99 amino acids. All patients have the same mutation and no other mutation could be found in the RSK2 gene. Thus, this novel mutation is likely to be responsible for the unusual clinical presentation in this family that includes full phenotypic expression in females and unique brain MRI abnormalities. The pathological function for the mutant to result in this clinical presentation as well as genotype/phenotype correlation awaits further clarification.

Detection of PLP duplications in Pelizaeus-Merzbacher Disease: Comparison of multiple methodologies in measurement of gene copy number. *Q. Gao, V.C. Thurston, G.H. Vance, S.R. Dlouhy, M.E. Hodes.* Dept Medical & Molecular Gen, Indiana Univ school of Medicine, Indianapolis, IN.

Pelizaeus-Merzbacher disease (PMD) is a rare, X-linked neurological disorder characterized by dysmyelination of the central nervous system (CNS). The genetic etiology of this disease is associated with mutations in the gene encoding proteolipid protein (PLP), the most abundant protein in the CNS myelin. In addition to a wide spectrum of point mutations and frameshift mutations, duplications involving the entire PLP gene appear to be the major cause of PMD, accounting for 50%-70% of cases with a family history of PMD or X-linked spastic paraplegia (SPG2). Techniques used to identify duplications of PLP include Southern blot, densitometric RFLP, semiquantitative comparative multiplex PCR (CM-PCR) and fluorescence in situ hybridization (FISH). However, each technique has certain limitations in clinical testing for PMD. In the current study, we investigated a relatively new technology, real-time PCR for the detection of duplications of the PLP gene. Forty-two patients with clinical and neurological features of PMD/SPG2 were included in this study. The data generated from real-time PCR was compared blindly to data obtained from FISH and CM-PCR. All three methods identified PLP duplications in sixteen of the samples and one sample had a deletion of the PLP gene. One sample was duplicated by real-time PCR, but not by FISH and CM-PCR. Possible explanation for this discrepant sample might be the detection of low-level mosaicism by the extremely sensitive real-time PCR. Twenty-four samples were negative for duplications of PLP by all three methods. The advantages, limitations and clinical applications of each method are compared and discussed.

Characterisation of mutations in patients with low level mosaicism by fraction collection of dHPLC separated heteroduplex peaks. *P. Emmerson¹, J. Maynard¹, R. Butler², J.R. Sampson¹, J.P. Cheadle¹.* 1) Institute of Medical Genetics, University of Wales College of Medicine, Cardiff, UK; 2) Medical Genetics Service for Wales, University Hospital of Wales, Cardiff, UK.

Somatic mosaicism is a frequent phenomenon in mendelian disorders that exhibit a high proportion of new mutations. However, mutant alleles present at low frequency may escape detection. We have previously shown that denaturing high-performance liquid chromatography (dHPLC) can detect TSC1 and TSC2 mutations in tuberous sclerosis patients with low-level somatic mosaicism, even when direct sequencing can not identify the causative lesion. Characterisation of these mutations currently involves extensive sequencing of cloned products. To overcome this limitation, we have utilised dHPLC with an in-line fraction collector to isolate low-level heteroduplex peaks which can be sequenced directly to reveal the mutation. We have successfully applied this technique to identify the mutations 2724-1G>C in TSC1 and 1772del4bp in TSC2 which were present in only 6 percent of the patient's peripheral blood lymphocytes.

A new homozygous mutation of the Lipoprotein Lipase Gene: (D174V) The first Lebanese mutation. M.

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Many diseases including chylomicronemia, pancreatitis and atherosclerosis are directly or indirectly related to abnormalities in the lipoprotein lipase (LPL) function. This enzyme plays a major role in lipid metabolism by regulating the catabolism of triglycerides rich lipoprotein particles. The human LPL gene is located on chromosome 8p22. Several mutations of LPL have been identified so far and influence LPL function in different ways. We report the first mutation of the LPL gene found in the Lebanese population. We investigated the molecular defect resulting in fasting hyperchylomicronemia, and recurrent severe pancreatitis in a Lebanese patient of consanguinous parents by sequencing the 10 exons of LPL. We identified a new homozygous missense mutation in exon 5 resulting in the substitution of Val for Asp at position 174 of the mature LPL protein. Mutations in exon 5 are the most commonly reported cause of LPL deficiency. As no enzyme restriction site was changed by this mutation we designed modified primers mismatching with the normal LPL gene and introducing a unique BclI restriction site in the normal sequence that is not generated when the mutation is present. This PCR-mediated site-directed mutagenesis allowed segregation study of the mutation in the proband's family and rapid screening of 100 Lebanese normolipidemic patients in which the mutation was not found. The identification of this new mutation and this rapid screening method will help diagnosis of LPL deficiency in the Lebanese population, consanguinous and known for its high dyslipidemia incidence. The possibility of a founder effect for this LPL mutation and its link with the Lebanese founder mutation (C660X) of the LDLR (low density lipoprotein receptor gene), are under investigation. In conclusion we report a new mutation (D174V) in LPL gene and a rapid screening method. This is the first mutation of the LPL gene found in the Lebanese population.

Program Nr: 2205 from 2002 ASHG Annual Meeting

Analysis of eight exons of the CFTR gene in 65 Iranian Cystic Fibrosis patients by DHPLC and sequencing. *E. Elahi*^{1, 2}, *B. Alinasab*², *G. Esson*², *F. Ghassemi*¹, *M. Houshmand*⁴, *A. Khodadad*³, *P. Oefner*², *F. Reihani-Sabet*¹, *M. Ronaghi*², *M. Shahabi*¹, *Y. Thorstenson*². 1) Dept Biological Sci, Tehran University, Tehran, Iran; 2) Stanford Genome Technology Center, Stanford University, Palo Alto, CA; 3) Pediatric Polyclinic, Children's Hospital Medical Center, Tehran University, Tehran, Iran; 4) National Research Center of Genetic Engineering and Biotechnology, Tehran, Iran.

Exons 3,4,6b,10,12,17b,19 and 20 and neighboring intron sequences of the Cystic Fibrosis Transmembrane Regulator (CFTR) gene were analyzed in 65 Iranian Cystic Fibrosis patients by Denaturing High Pressure Liquid Chromatography (DHPLC). The PCR products of exons harboring mutations were sequenced by the dideoxy protocol. The sequences were compared to wild type sequences using the Sequencher sequence analysis software. In the eight exons analyzed, 24 different variations were detected. Five of these appear to be changes not previously reported: N408®G (E92E), deletion of GATT between N867-33 and N867-6 (deletion of one of seven repeats in intron 6b), N1655T®A (F508I), N1716+8A®G, and N3811T®G (L1227V). We are in the process of completing mutation analysis in all the exons of the Iranian patients.

Low proportion of dystrophin gene deletions among Filipino Duchenne and Becker Muscular Dystrophy patients. *E.C. Cutiongco¹, B.C. Cavan¹, E.C. Abaya¹, C.L. Silao¹, C.D. Padilla¹, H. Nishio², M. Matsuo².* 1) Institute of Human Genetics, Manila, Philippines; 2) International Center for Medical Research, Kobe University Graduate School of Medicine, Japan.

Duchenne muscular dystrophy (DMD), an X-linked progressive neuromuscular disorder, affects 1 in 3500 male livebirths. The milder allelic form, Becker muscular dystrophy (BMD), occurs in 1 in 20,000. Both are caused by mutations in the dystrophin gene. In DMD, affected individuals have little or no functional dystrophin while BMD cases may have a partially functional dystrophin giving rise to milder clinical manifestations. Mutations causing DMD/BMD are deletions of one or more exons of the dystrophin gene, exon duplications, or point mutations. The proportion of deletions among mutant dystrophin alleles in North American and European studies is 55-65% while a lower proportion (37%) of dystrophin gene deletions among Israeli DMD and BMD patients has been described. Other Asian populations have likewise shown a lower proportion of deletions among mutant dystrophin: 40-43% in Japanese and 45-50% in Chinese populations. A racial difference in the proportion of deletions may exist. We examined DNA samples of 35 unrelated Filipino patients diagnosed with DMD and BMD. Of these patients, 13 have been included in a previous report; this is an extension of that study. Deletions were detected using the multiplex DNA amplification procedure which permits rapid identification of 80-90% of all dystrophin gene deletions. Our results show that 8 of the 35 patients (22.8%) have deletions in at least one of the 23 exons of the dystrophin gene examined using multiplex PCR. This proportion of dystrophin gene deletion is the lowest reported so far in comparison to other populations. This low proportion makes diagnosis using the current PCR techniques particularly difficult. We also found that deletions among Filipino DMD/BMD patients were more common in the 5' end (62.5%) than in the central rod domain (37.5%). The findings suggest the presence of genetic variability among DMD/BMD patients in different populations.

Improvement of the scanning of the *CFTR* gene by multiplexing DHPLC analysis. *C. Ferec*^{1,2,3}, *K. Rouault*², *M.P. Audrezet*^{1, 2, 3}, *C. Le Marechal*^{1, 2, 3}. 1) Lab de Biogenetique, Universite de Bretagne Occidentale, Brest, France; 2) INSERM EMI 0115, molecular and epidemiological genetic, Brest, France; 3) CHU Brest, France.

We have recently shown that DHPLC is a powerful scanning technique for identification of point mutations in the *CFTR* gene (Le Marechal et al. 2001). Up to now all mutations tested were detected. To improve the speed of the complete analysis of the 27 exons we have developed a multiplexe strategy. Taking into account the oven temperature deduced from the melting profile of each amplicon, and the acetonitril concentration for their elution, we designed 7 multiplexe PCR. We report the PCR conditions and DHPLC settings to achieve these analysis. Only 20 DHPLC injections are now necessary for studying the coding sequence of the *CFTR* gene. The multiplexing of two amplicons can save 25% analysis time and allows to reduce the cost bellow one US dollar per amplicon scanned. One need rapid (few days) and accurate technique to scan the complete coding sequence in emergency situation such as prenatal diagnosis requested after the discovery of a hyperechogenic bowell during ultrasonnd screening. Quick typing with specific tests looks for only 30 most frequent mutations compared to more than one thousand described in the CF database (www.genet.sickkids.on.ca). The residual risk for CF in a couple with one unidentified allele is decreased from 1/10 to 1/60 if the technique used has 98% detection rate which is DHPLC performance.

Program Nr: 2208 from 2002 ASHG Annual Meeting

Validation of the novel WAVE-MD™ System technology for Mutation detection screening by DHPLC for Cystic Fibrosis. *C. Griffiths¹, G. Restagno², S. Papadimitriou¹, J. Walter¹*. 1) Transgenomic Ltd., UK; 2) Dipartimento di Patologia Clinica, Settore Genetica Molecolare Azienda Sanitaria Ospedaliera O.I.R.M.S.ANNA, Torino, Italy.

The WAVE-MD™ System is a new platform for mutation detection by Denaturing High Performance Liquid Chromatography (DHPLC). It can be used to identify known mutations or to detect unknown mutations, and offers a number of key innovations to the previously reported methods. The primary advantages of the WAVE -MD™ System are the disposable cartridge used for DNA analysis underlying the DHPLC technique, simplification of the gradient with regards to method development, and an advanced software incorporating automated mutation calling. We detail here initial experiments to validate the technology platform by screening a panel of previously characterised mutations and demonstrate the same high efficiency of mutation detection as reported with the conventional instrumentation and cartridge technology. In addition, applications specific work is performed to screen the CFTR gene, emphasising the transferability of previously characterised DHPLC methods for detection of specific mutants and providing data as to the accuracy of the mutation calling capability.

An homozygous mutation in the Fukitin-related protein gene(FKRP) causing a severe MDC1C form with mental retardation and cerebellar cysts:(Founder affect in Tunisian families). *F. FAKHFAKH¹, N. LOUHICHI¹, C.*

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The congenital dystrophies(CMD)are a heterogeneous group of autosomal recessive disorders presenting in infancy with muscle weakness, contractures and dystrophic change on muscle biopsy.Approximately 40%of patients with CMD have a primary deficiency (MDC1A)of laminin alpha 2 chain (merosin) due to mutations in LAMA2 . In addition, a secondary deficiency of laminin alpha2 is associated with some CMD forms, including MDC1B, MEB, FCMD and MDC1C mappedrespectively to chromosomes 1q42, 1p3, 9q3 and 19q13.Mutations in the FKRP gene have been identified in patients affected with a severe form of MDC1C, which is characterized by inability to walk, muscle hypertrophy ,marked elevation of serum creatine kinase and normal brain structure and function.We have performed immunohistochemical and genetic analyses for five patients belonging to five consanguineous Tunisian CMD families presenting muscle hypertrophy, marked elevation of serum creatine kinase,cerebellar cysts and mental retardation. Immunohistochemical analysis revealed a partial deficiency of merosin in all the patients.Linkage analysis showed an exclusion of FCMD, MEB, MDC1A and MDC1B loci but linkage to MDC1C locus.Mutation screening of the whole FKRP coding region revealed a new homozygous C1363A (Ala/Asp)missense mutation in the five patients. This mutation was not found in more than 200 control chromosomes.The presence of the same mutation in these 5 patients suggests a founder affect which has been confirmed by haplotype analysis. This is the first homozygous mutation in FKRP causing CMD with mental retardation and brain abnormalities.

Comparison of two methods for routine accurate determination of the two mutations in the *IKBKAP* gene causing Familial Dysautonomia. A. Blumenfeld¹, M. Idelson¹, L. Reznik¹, I. Bejarano-Achache¹, C. Maayan¹, F.B. Axelrod², A. Yakir³, A. Bessudo³, Y. Furman³, N. Navot³. 1) Hadassah Univ Hosp, Jerusalem, Israel; 2) NYU Med Center, New York; 3) Savyon Diagnostics Ltd, Ashdod, Israel.

Familial dysautonomia (FD) is an autosomal recessive disorder, caused by poor development, poor survival, and progressive degeneration of the sensory and autonomic nervous system, affecting many other body systems. FD is inevitably fatal, with only 50% of patients reaching the age of 30. The frequency of the disease among Ashkenazi Jews is about 1:3,700, which corresponds to a carrier rate of 1:32. All cases were due to two mutations in the *IKBKAP* gene: one in intron 20 with a frequency >99%, and the other in exon 19, with a frequency of less than 1%. The disease was also observed among non-Ashkenazi Jews, mainly among Sephardic Jews. All but one non-Ashkenazi patients and carriers have the major mutation. Detailed haplotype analysis of these individuals showed that they are all of the same ancestral origin, and share the same haplotype over several centimorgans. One non-Ashkenazi carrier has the minor mutation / typical minor haplotype. To date, no other mutations in the *IKBKAP* gene, causing FD, have been identified.

We have developed two simple screening methods for these mutations. The first is based on digestion of PCR-amplified FD gene fragments with a restriction enzyme followed by gel electrophoresis, and the other - on a single nucleotide primer extension reaction, followed by ELISA in a kit format. Comparison of the two methods on the same set of samples provided identical results. The results correspond to those obtained by haplotype analysis.

Lately, there has been a dispute regarding the actual frequency of the minor FD mutation: though observed in less than 1% of FD families, the possibility of a higher carrier frequency of this mutation in the Ashkenazi Jewish population, has been raised. The availability of our two methods for detection of mutations causing FD, can serve as an important tool in resolving this dispute. These detection methods allow fast, precise and cost-effective population screening tool for this devastating disorder.

A family with episodic ataxia type 2; no evidence of genetic linkage to the CACNA1A gene on chromosome

19p13. *Y. Nagao*^{1, 2}, *H. Hirose*², *J. Takita*², *T. Arayama*³, *T. Igarashi*², *Y. Hayashi*². 1) Department of Pediatrics, Social Health Insurance Medical Center, Tokyo, Japan; 2) Department of Pediatrics, University of Tokyo Hospital, Tokyo, Japan; 3) Department of Pediatrics, Kikkoman Hospital, Chiba, Japan.

Episodic ataxia type 2 (EA2) has been reported to result from mutations in the CACNA1A gene, located on chromosome 19p13. We report a family with episodic ataxia, clinically indistinguishable from EA2, that was not caused by CACNA1A gene mutation. The proband is a 10 y-old boy, who has had 6 cerebellar ataxic attacks since 8 y. His attacks occurred almost monthly, lasting for 2 to 3 days. He was treated successfully with acetazolamide. His maternal grandmother, mother and twin brother had similar attacks since they were 50 y, 34 y and 10 y, respectively. The symptoms in his grandmother improved gradually without medication. His mother and twin brother took acetazolamide with a good response. We examined the CACNA1A gene for this family but did not detect any mutations. Furthermore, there was no evidence of genetic linkage between the CACNA1A gene and the symptomatic patients in this family. These suggest that the cause of EA2 can be heterogeneous, that is, defects of genes other than CACNA1A might also be the cause of EA2.

Comprehensive screening of the RYR1 gene by denaturing high-performance liquid chromatography. S.

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Malignant Hyperthermia (MH) is a life-threatening hypermetabolic crisis following exposure to commonly used anesthetics. In the majority of cases, MH results from a defect in regulation of calcium release in skeletal muscle due to dominant mutations in the calcium channel of the sarcoplasmic reticulum, ryanodine receptor (RYR1 at 19q13). Because of the large size of the RYR1 gene, most genetic screening studies have targeted few known mutational hot spots and as a result mutations were identified in only about 25% of studied patients. The aim of this study was to develop an efficient method of finding most, if not all mutations in the RYR1 gene. We analyzed the entire coding region of the RYR1 gene in eight MH patients using denaturing high-performance liquid chromatography (DHPLC). One known mutation and five novel amino-acid substitutions were found in the RYR1 gene. The four amino acid changes were considered as likely candidate mutations for MH, based on their cosegregation with MH, absence among 50 healthy controls and location at conserved residues within RYR1 proteins. Two novel candidate mutations were located outside of known mutational hot spots. Our preliminary data suggest that mutations in the RYR1 gene are distributed throughout the gene and the efficiency of mutation detection in patients with MH can be increased from 25% to greater than 60% with the use of highly sensitive DHPLC screening technique.

Mutation Analysis of Sex Determined Genes in Korean Patients with Sex-Reversal Disorders. *S.C. Jung¹, K.S. Lee¹, Y.J. Lee¹, J.A. Park², J.S. Lee².* 1) Division of Genetic Disease, Korea National Institute of Health, Seoul 122-701, Korea; 2) Department of Pediatrics (Medical Genetics), Yonsei University College of Medicine, Seoul 120-752, Korea.

The XY chromosome configuration determines male and the XX female, but in sex reversal syndrome a male with XY will develop female sex organs or a woman with XX will have male characteristics. All of steps of sex differentiation are under genetic control. Therefore mutations of the corresponding genes can cause the failure of sex differentiation. Genomic DNA was extracted from the lymphocytes of patients and their family, with sex-reversal disorders who presented with 46, XY, phenotypic female or 46, XX, phenotypic male. We have performed molecular studies of sex determined genes; *sex-determining region Y (SRY)*, *anti-Müllerian hormone (AMH)*, *steroid 21-hydroxylase*, *androgen receptor (AR)*, *steroidogenic factor-1 (SF-1)*, *doublesex*, *mab-3 related in testis-1 (DMRT-1)* and *Wnt-4*. Using PCR and direct-sequencing method, we identified 2 insertion or deletion mutations in the whole sry open reading frame (ORF), 8 missense mutations - Pro31Leu, intron2 splice site, Ile173Asn, Val282Leu, Gly292Ser, Leu308insT, Gln319Stop, Arg357Trp - in *steroid 21-hydroxylase* gene. In addition, 3 mutations in *AR* gene and duplication of *Wnt-4* gene were identified. For *AR* gene, there were a single nucleotide C deletion at amino acid 39, resulting in frameshift mutation and two missense mutations; Leu57Gln, Ala596Thr. Ala596Thr mutation was found in two brother patients, whose mother showed heterozygosity at the same position. In *SF-1* and *DMRT-1* genes, no mutations have been detected yet. We are going to analyze more candidate genes to find causative mutations of sex reversal disorders.

Spontaneous tandem-base mutations in mouse and man: rare occurrence in the germline and similar mutation types in the soma. K.A. Hill, J. Wang, K. Farwell, S.S Sommer. Dept Molecular Genetics, City of Hope Natl Med Ctr, Duarte, CA.

The frequency, pattern and spectrum of spontaneous tandem-base mutations (TBM) have not been well characterized. We previously analyzed spontaneous TBM in a transgenic mouse model for detection of *in vivo* somatic and germline mutations (Big Blue). The frequency of TBM showed age and tissue specific differences. TBM had a unique pattern of mutation types with a predominance of G:C to T:A (70%) and specifically GG to TT and GG to AT mutations (58%). To explore the relevance of spontaneous TBMs, we sequenced 2,593 additional mutants from 11 tissues, tripling the sample size. Forty-four TBM were identified. The previous findings were confirmed and several new observations were made. TBM were most frequent in the kidney (3.45×10^{-6}), accounting for 7.6% of all mutational events in kidney. TBM were also frequent in liver and adipose and infrequent in most other tissues. The frequency of TBM in liver increased dramatically with age. TBM frequency in kidney is at least 60-fold higher than in the germline. TBM are also rare in the human germline (one occurrence in 5,142 germline mutations present in human Factor IX, Factor VIII, HNPCC, and P53 mutation databases). Somatic TBM were more frequent in humans (P53, supF, HPRT and APRT databases). The pattern of somatic TBM mutations in the P53 gene was similar, although not identical in mice and humans (TBM related to UV-induced skin cancer were excluded). In general, the types of TBM were similar in mice and humans except for an excess of CA to TG TBM in humans. The data indicate that TBM are a significant fraction of mutations in certain somatic tissues and that the unknown endogenous mechanisms for TBM may be similar in mice and man.

Routine analysis of the CFTR IVS(8)T polymorphism discloses two pathogenic mutations. *C. Kraus¹, L. Naehrlich², S. Mattes³, A. Reis¹.* 1) Institute of Human Genetics, University of Erlangen-Nuremberg, Erlangen, Germany; 2) Pediatric Hospital, University of Erlangen-Nuremberg, Erlangen, Germany; 3) Pediatric Hospital of the Thuringen-Clinic, Salfeld, Germany.

Cystic fibrosis (CF) is the most common autosomal recessive disorder in the Caucasian population, caused by hundreds of mutations in the cystic fibrosis conductance transmembrane regulator (CFTR) gene. Routinely, we use an Oligonucleotide ligation assay (Applied Biosystems) to screen for 31 mutations in the CFTR gene, including 24 of the most common. Additionally, CFTRdele2,3(21kb), which occurs with a frequency of 1% in our population is analyzed by multiplex PCR. The application of these methods allows us to identify both disease causing mutations in 111 (87%) out of 127 proven CF patients. With regard to CBAVD the polymorphism IVS(8)T were examined by PCR/restriction analysis and subsequent PAGE. Generally, the poly-T tract in intron 8 exists in three variants, 5T, 7T, and 9T. The 7T and 9T variants generate a predominantly normal transcript, whereas the 5T allele reduces the level of functional CFTR and is associated with an inherited form of infertility in males (CBAVD). The existence of further allelic variants (3T, 6T and 8T) was shortly mentioned by Doerk et al. (2001). From our CF patients four out of 127 exhibited an abnormal migration pattern in the PAGE of IVS(8)T. In two patients PAGE results suggested a 6T allele in addition to a 9T and 7T allele, respectively. Sequencing, however, disclosed the 6T allele of one patient as a 7T allele carrying a splice site deletion (IVS8-1delG). In the other two patients PAGE displayed a 7T and 9T allele with an abnormal heteroduplex formation. In these cases a IVS8-2A->C transversion was discovered in cis with the 7T allele. By carefully evaluating the migration patterns we were able to identify the second disease allele in three additional patients. Thereby both disease causing mutations could be identified in 90% versus 87 % of our patients analyzed. We therefore conclude that any atypical T variant has to be carefully analyzed for the underlying mechanism.

Identification of a Putative New Hypochondroplasia Mutation in the Intracellular Juxtamembrane Domain of

FGFR3. *F.G. Lux III¹, A.S. Aylsworth², S.H. Blanton³, T.E. Kelly³, G.A. Bellus⁴ and The Genentech Hypochondroplasia Study Group.* 1) Dept Biol, Lander Univ, Greenwood, SC; 2) Dept. Pediatrics & Genetics, Univ. North Carolina, Chapel Hill, NC; 3) Dept. Pediatrics, Univ. Virginia, Charlottesville, VA; 4) Depts Dermatology, Pediatrics and Human Medical Genetics Program, University of Colorado, Denver, CO.

Dominant point mutations in the fibroblast growth factor receptor 3 (FGFR3) gene are known to cause several different forms of human dwarfism. Hypochondroplasia is one of the milder forms and it is estimated that 50 - 70% of all cases are caused by 2 point mutations (C1620A & C1620G) that result in an N540K amino acid substitution in the exon 13 proximal tyrosine kinase domain. There have been reports of rare cases of hypochondroplasia resulting from other FGFR3 amino acid substitutions occurring in exon 13 (N5540T, N540S, I538V), exon 15 (K650N, K650Q) and exon 9 (N328I). The underlying genetic etiologies in the remaining 30 -50% of hypochondroplasia diagnoses remain unknown and some genetic heterogeneity has been demonstrated. We have begun a screen for novel FGFR3 mutations employing the WAVE DNA Fragment Analysis System (Transgenomic, Santa Clara, CA) in hypochondroplasia patients in whom known FGFR3 mutations have been excluded. An examination of up to 12 exons from 42 individuals has resulted in the identification of a number of putative new polymorphisms or mutations in several FGFR3 exons. One putative new mutation (C1346T) results in a P449L substitution in exon 11, which encodes the intracellular juxtamembrane domain. This proline codon is highly conserved among all FGFR genes in chordates. We speculate that this amino acid substitution causes constitutive activation of FGFR3 and results in a hypochondroplasia phenotype.

Determination of the mutation spectrum in Dutch patients with Maturity Onset Diabetes of the Young (MODY).

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Type 2 diabetes shows multifactorial aetiology with strong evidence for an inherited component. Several monogenic, highly penetrant type 2-like diabetes subtypes have been defined, accounting for about 5% of the Dutch diabetic patients. Among these, the most important is Maturity Onset Diabetes of the Young (MODY). MODY shows an autosomal dominant pattern of inheritance and can be divided in at least 7 subtypes (MODY1-7), each subtype being caused by mutations in a specific gene. The unambiguous molecular diagnosis of the specific MODY subtype allows the early diagnosis of diabetes that can help to prevent the development of diabetic complications. Also does it allow risk assignment for diabetic complications to particular diabetic subtypes. Presymptomatic testing in relatives at-risk allows an early diagnosis of their diabetes and close monitoring and treatment will help to prevent the development of diabetic complications. The DNA of a group of 81 patients, fulfilling the criteria for MODY, e.g. autosomal dominant pattern of inheritance and early-onset type 2 diabetes, was scanned by automated direct sequence analysis for the presence of mutations in the HNF-1alpha, glucokinase, HNF-4alpha, IPF-1, and HNF-1beta genes known to be involved in MODY types 1-5. In 28% of the patients a mutation in one of these genes was found. In 19% the HNF-1alpha gene was mutated, while in 5% a mutation in the glucokinase gene was found and in another 5% a mutation in the HNF-4alpha gene was present. No mutations in either the HNF-1beta or IPF1 gene were detected. *This study is financially supported by the Diabetes Fonds Nederland.*

Molecular investigation of Alagille syndrome in patients candidated to liver transplantation. *G. Massazza¹, P. Stroppa², M. Iascone¹, M. Poma¹, A. Sonzogni¹, A. Parma¹, B. Gridelli³.* 1) UO Anatomia Patologica; 2) UO Pediatria; 3) Centro Trapianti Fegato, Ospedali Riuniti, Bergamo, Italy.

Alagille syndrome (AGS) is an autosomal dominant developmental disorder, characterised by bile ducts paucity and resultant liver disease in association with cardiac, skeletal, ocular and craniofacial abnormalities. It is caused by deletions or mutations in the Jagged 1 (JAG1) gene located on chromosome 20p12, which encodes a ligand for Notch family receptors. In 15% of the AGS patients, hepatic involvement progresses to liver failure that requires organ transplantation. AGS shows highly variable penetrance, and diagnosis in mildly affected patients can be difficult. The JAG1 gene spans 36kb and consists of 26 exons, encoding a 5,5 kb transcript. The aim of our study was the screening of JAG1 alterations in paediatric patients with unexplained cholestasis, waiting for liver transplantation. Seven patients with strong clinical evidence of Alagille syndrome (AGS) and 9 children with unexplained cholestasis with some features of Alagille (p-AGS) together with their respective parents were enrolled in the study. The overall median age was 12,5 months (range: 50days-11years). We performed the screening of 20p12 deletions by STRP analysis, mapping into the region. SSCP analysis of JAG1 gene was carried out in no-deleted patients. We did not find any deletions but we detected 15 DNA alterations in JAG1 by SSCP analysis. DNA sequencing revealed presence of 2 nonsense, 2 missense, 7 silent and 4 intronic sequence variations. The sequence variations, causing an aminoacid substitution or truncation of the protein, were tested in the parents and in fifty unrelated and healthy controls. R235X and Q304X were de novo, instead P871R and R937Q were paternally inherited. The missense mutation P871R was a known polymorphism while R937Q was not found in 100 control chromosomes. In conclusion, we identified 3 mutations among the 7 AGS patients (43%) and no significative alterations in pAGS patients.

Identification of Novel CLN2 Mutations Reveals Canadian-Specific NCL2 Alleles. *W. Ju^{1,2}, R. Zhong⁴, S. Moore⁵, D. Moroziewicz^{1,2}, J. Currie², P. Parfrey⁵, W.T. Brown², N. Zhong^{1,2,3}.* 1) SCL-Molecular Neurogenetic Diagnostic Laboratory; 2) Dept. Human Genetics, New York State Inst Basic Res, Staten Island, NY; 3) Dept. Neurology, SUNY Downstate Health Center, Brooklyn, NY; 4) Stuyvesant High School, New York, NY; 5) Clinical Epidemiology Unit, Health Sciences Center, Memorial University of Newfoundland, St. Johns, NF, Canada.

Late-infantileonset neuronal ceroid lipofuscinosis (LINCL or NCL2) is among the most common childhood neurodegenerative disorders. It has been reported in the Canadian population. Among 23 Canadian families studied with molecular approaches, 20 were confirmed as having NCL2, the late-infantile onset NCL underlied by gene CLN2. The mutation G284V, which is found only in Canadian NCL2 and accounts for 55% of the NCL2 families and 32% of the mutant alleles, indicates that G284V presents as a founder mutation. Along with two other common CLN2 mutations IVS5-1GC, which accounts for 25% (10/40), and R208X (636CT), which accounts for 10% (4/40), of Canadian mutant NCL2 alleles, G284V should be applied to clinical molecular diagnosis for Canadian NCL2. As a result, molecular study of the three mutations for Canadian LINCL would detect 85% of NCL2 families and 67% of mutant NCL2 alleles. In addition, several novel mutations that are clustered in the distal coding sequence of the CLN2 gene have been identified in Canadian NCL2 alleles. The G284V mutation appears to interfere with normal protein folding and thus might cause the loss of function of the lysosomal enzyme tripeptidyl peptidase1 (TPP1), which is deficient in individuals with NCL2.

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Characterization of a Bgl I RFLP in DMPK gene and its implications in molecular diagnosis of Myotonic dystrophy. *L.N. Hjelm, K. Muralidharan.* Department of Pediatrics, Division of Genetics, Emory University School of Medicine, Atlanta, GA 30322.

Myotonic Dystrophy (DM) is an autosomal dominant muscle disorder caused by expansion of the CTG trinucleotide repeats in the DMPK gene. Point mutations, deletions, insertions in the DMPK have not been reported. PCR and Southern Blot analysis are used to determine CTG repeat sizes in DMPK gene. Southern blot analysis is used to detect large expansions not detectable by PCR amplification. We detected a novel Bgl I polymorphism in DMPK gene that could be mistaken for an expansion of CTG repeats. It was detected in a patient who had two normal size alleles in PCR but showed an 'expanded' allele in Southern analysis. Testing with other restriction enzymes did not detect an expanded allele. This variation does not appear to be pathogenic as it was also found in the father who was not symptomatic. A Hha I polymorphism in intron 5 and a Hinf I polymorphism in intron 9 of DMPK gene have been reported earlier. The site of the Bgl I polymorphism lies in the DMAHP gene at the 3' end of DMPK gene. This polymorphism could be source of false positive result in molecular diagnosis of DM using Bgl I restriction digestion.

Spectrum of mutations in the CLN6 gene. *C.A.F. Teixeira*^{1,2}, *J.E. Espinola*³, *C.J.P Bessa*², *A. Guimares*^{4,5}, *M.C.S Miranda*^{2,6}, *M.E. Mcdonald*³, *M.G. Ribeiro*^{2,6}, *R.M.N. Boustany*¹. 1) Pediatrics and Neurology, Duke University, Durham, NC; 2) Departamento de Neurobiologia Genetica do Instituto de Biologia Molecular e Celular da Universidade do Porto, Portugal; 3) Molecular Neurogenetics Unit, Massachusetts General Hospital, Charlestown; 4) Unidade de Neuropatologia, Hospital Geral de Santo Antnio, Porto, Portugal; 5) Departamento de Patologia e imunologia, Instituto de Cincias Biomdicas Abel Salazar, Universidade do Porto, Portugal; 6) Unidade de Enzimologia do Instituto de Genetica Medica do Porto, Portugal.

The neuronal ceroid lipofuscinoses (NCLs) are a genetically and clinically heterogeneous group of autosomal recessive neurodegenerative diseases. This group of diseases is characterized by the accumulation of autofluorescent lipopigment in various tissues, and by progressive cell death in brain and retina. Recently the CLN6 gene causing variant late infantile NCL in Costa Rican and Portuguese patients was identified (pLINCL; OMIM 601780). The number of mutations found suggests that the CLN6 gene may be a highly mutable gene. We summarize previously described mutations, and describe three novel mutations in the CLN6 gene. An American patient was found to be heterozygous for a 4bp insertion in exon 3. The mutation in the other allele consists of a point mutation in exon 7. The third novel mutation was identified in a Portuguese patient heterozygous for the I154del Portuguese CLN6 mutation. This third mutation is a double mutation of a 4pb deletion and a 1bp deletion in exon 7. Three Portuguese patients with a clinical profile similar to CLN6 patients, but no defects in the CLN6 gene or any other known NCL gene were identified. Our work has led to these conclusions: (1) variant late infantile Batten disease in Portugal is genetically heterogeneous (2) the CLN6 gene is mostly likely a highly mutable gene and (3) the I154del accounts for the CLN6 defect in 79% of Portuguese alleles studied (4) three variant late infantile Portuguese patients may have defects in a new NCL gene.

SHOX deletions and point mutations in 56 patients with short stature. *L. Stuppia*^{1,2}, *G. Calabrese*^{1,3}, *E. Morizio*^{1,3}, *V. Gatta*¹, *S. Pintor*¹, *P. Guanciali Franchi*^{1,3}, *D. Fantasia*^{1,3}, *G. Palka*^{1,3}. 1) Dept di Science/Biomed, Univ G D Annunzio, Chieti, Italy; 2) Istituto di Citomorfologia Normale e Patologica, CNR, Chieti, Italy; 3) Servizio di Genetica Umana, Ospedale di Pescara, Italy.

Stature is the result of interactions of several factors including those of genetic origin. About 3% of people suffer of short stature and in most of them the cause is unknown. Recently, the SHOX gene (Short Stature Homeobox containing gene), mapped on the pseudoautosomal region (PAR1) of the X and Y chromosomes, was specifically associated with the short stature of patients with Turner syndrome and those with Leri Weill dyschondrosteosis (LWD). Few data are reported about the relationship between SHOX mutations and idiopathic short stature. We investigated 56 patients with short stature of unknown origin, using FISH analysis for the detection of SHOX deletions, and direct sequencing of exons 2-6a for the screening of point mutations. With these approaches, we detected SHOX deletion in 4 cases (7.1%) and SHOX point mutation in 3 patients (5.3%). The mutations consisted of a C-G transversion on nucleotide 548 (C548G) within exon 3 leading to an Arg-Gly change within the SHOX homeodomain. The prevalence of SHOX deletions/mutations in this study are higher in respect to those present in the literature. These discrepancies are likely due to the limited number of reported cases and to the different techniques employed, particularly to the use of direct sequencing analysis, more sensitive than SSCP in detecting point mutations. Two patient with SHOX deletion and one with point mutation were submitted to rhGH treatment, showing a good response. Thus, it is possible to recommend the use of rhGH therapy in patients with SHOX rearrangements, since these have to be considered as Turnerian patients, who show a positive response to this treatment.

Maple syrup urine disease: A Common Novel Mutation Identified Among Affected Individuals of the Philippines.

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BACKGROUND OF THE STUDY: Defective function of the branched chain α -ketoacid dehydrogenase complex [BCKD] produces the clinical phenotype of Maple syrup urine disease [MSUD]. Several causal mutations have been identified in the 3 catalytic components comprising this enzyme complex, one of which is the dihydrolipoyl transacylase [E2] subunit. A study to determine the molecular basis of MSUD was carried out on 15 subjects belonging to 13 unrelated families from the Philippines. **METHODS:** The approach consisted of: (1) isolating DNA from peripheral blood of clinically diagnosed MSUD affected individuals and a normal control; (2) performing PCR amplification of the entire E2 subunit of the BCKD enzyme complex in both patient and normal control DNA; and (3) subsequent direct sequence analysis of the regions flanking the E2 gene defect. **RESULTS:** We identified the same novel molecular lesion in a large proportion of the subjects studied (10 of 15) with 7 of them, belonging to 5 unrelated families, being homozygous for the mutation. Two of the 3 compound heterozygote patients presenting with this common novel mutation had novel missense mutations. Another previously reported missense mutation was also found in 2 other compound heterozygotes. **CONCLUSION:** This study provides the molecular basis for understanding this genetic disorder among Filipino MSUD patients. The novel mutation in our subjects suggests that identification of this mutation will facilitate, if it is an option, prenatal diagnosis of affected families. Effective carrier detection may decrease the incidence and/or the morbidity associated with delayed diagnosis of this disorder among future Filipino MSUD patients.

***NROB-1* Intragenic Inversion Causes Adrenal Hypoplasia Congenita (AHC).** *Y. Zhang, U. Bhardwaj, B.H. Huang, M. Karpodinis, E.R.B. McCabe.* Departments of Pediatrics and Human Genetics, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095-1752.

X-linked Adrenal Hypoplasia Congenita (AHC) is a rare developmental disorder of the adrenal cortex. It is caused by molecular defects in the *NROB1* gene, which result in primary adrenal insufficiency and Hypogonadotropic Hypogonadism encoding DAX1 (HH). Most commonly, mutations of the *NROB1* gene include frameshifts and single nucleotide substitutions or large intergenetic contiguous gene deletions. Although more than 85 such mutations have been reported for *NROB1* to date, no large intragenic rearrangements have been documented. Here, we report the first case of AHC due to an interstitial inversion with breakpoints within exon 1 and the intron. The full coding region and the intron/exon boundaries of the *NROB1* gene were amplified into overlapping segments and sequenced. Dot blot hybridization was used to confirm the results. Using long PCR we were able to amplify exon 2. However, we could only amplify the 5 regions of exon 1 and the intron. The inconsistency of amplification in the 3 regions suggested the possibility of an inversion in this region. The exact breakpoints were precisely determined by PCR amplification and sequencing. Two pairs of primers were designed across the possible 5 and 3 junction, and subsequent PCR analysis indicated a nearly 3.5 kb inversion with a 3 contiguous 3 bp deletion (CTT) involving breakpoints within exon 1 and the intron: g.673_4161inv3488; 4162_4164delCTT. Knowing that intragenic inversions may occur within the *NROB1* gene raises important diagnostic issues. If one breakpoint is within an exon, as in our patient, the inversion should be identified when PCR amplification of the coding region fails. However, if breakpoints occur in the non-coding region, an inversion may be missed by PCR assays that are designed to analyze the coding region. If AHC is diagnosed clinically and no deletion or *NROB1* mutation is identified, then the possibility of an inversion should be considered.

Mutation Analysis and Genotype -Phenotype Correlation in a family with Mutations in *DNAH5* and Primary Ciliary Dyskinesia (PCD). M. Zariwala¹, P.G. Noone¹, A. Sannuti¹, S.L. Minnix¹, M. Hazucha¹, J.L. Carson¹, M.W. Leigh¹, H. Olbrich², H. Omran², M.R. Knowles¹. 1) Pulmonary / CF Research and Treatment Center, University of North Carolina at Chapel Hill, NC. USA; 2) Universitats-Kinderklinik, Freiburg, Germany.

Primary ciliary dyskinesia (PCD) is an autosomal recessive disorder associated with chronic sino-pulmonary infection. ~ 50% of the patients have situs inversus (SI) (Kartagener's syndrome). Clinical phenotype is caused by abnormalities of ciliary structure/function. *DNAH5* mutations have been shown to cause PCD and randomization of left/right asymmetry in patients with outer dynein arm defects (ODA). Recently, a homozygous splice mutation [IVS74-1G->C] in *DNAH5* has been identified in 2 siblings with PCD born to consanguineous couple (Olbrich et al. 2002). This mutation is predicted to cause loss of exon 75, and result in a truncated protein with loss of 72 amino acids. Both males (10 & 5 yr.) presented with recurrent oto-sinu-pulmonary infections. The younger sibling was born with SI and neonatal respiratory symptoms. This led to the diagnosis in him, plus an older sib without SI. Both had normal lung function. Carrier analysis was carried out in 13 family members. Parents, two maternal aunts, one maternal uncle and maternal grandfather harbored the mutation on one allele. The parents and unaffected sibling (7 yr.) were clinically normal. Nasal NO (nl/min; normal 36925) levels were severely reduced in patients (2 and 7), and intermediate in the parents (215, 212). Ciliary ultrastructure showed absent/shortened ODA (IDA) in both affecteds; the parents had mild shortening of DA (nm) relative to normal (IDA=12.2 vs 16.0, ODA 17.5 vs 21). This suggests obligate carriers may have sub-clinical biologic defects, such as lower than normal levels of nasal NO and slight shortening of DA. This family with clinical PCD provides an ideal opportunity to test the hypothesis that this splice-site mutation may results in a very reduced (or absent) level of expression of full-length transcript, and thus a truncated protein, manifested by shortened, stubby ODA. Quantitative estimates of *DNAH5* mRNA are underway. Supported by "CRU#RR00046".

A novel CACNA1A missense mutation in a family with EA-2 results in complete loss of P/Q-type calcium channel function. *K.A. Scoggan^{1,3}, J.E. McRory⁴, C.M. Santi⁴, T.P. Snutch⁴, J.H. Friedman⁵, D.E. Bulman^{1,2}.* 1) Ottawa Health Research Institute, Ottawa, ON, Canada; 2) Division of Neurology, Ottawa Hospital-General Campus, Ottawa, ON, Canada; 3) Nutrition Research Division, Health Canada, Ottawa, ON, Canada; 4) Biotechnology Laboratory, University of British Columbia, Vancouver, BC, Canada; 5) Brown University, Division of Neurology, Memorial Hospital of Rhode Island, Pawtucket, Rhode Island, NY, USA.

Episodic ataxia type-2 (EA-2) is an autosomal dominant neurological disorder that is usually caused by mutations in the CACNA1A gene that result in premature termination of translation. Affected individuals experience discrete episodes of cerebellar ataxia usually associated with migraine symptoms and respond to acetazolamide. We identified a 27 year old woman who began exhibiting signs of episodic ataxia at age 12. The "attacks" lasted about 20 minutes, were occasionally precipitated by stress, and sometimes preceded by a headache. Family history was positive for episodic ataxia, although a number of affected individuals in this family were originally thought to have some form of epilepsy. Initially the proband was placed on acetazolamide, but over time the attacks returned to their normal frequency. A combination of acetazolamide and valproic acid provided effective relief from attacks. At present her daughter, who appears to have similar symptoms, is responsive to acetazolamide treatment alone.

Mutational analysis of the CACNA1A gene revealed a novel missense mutation in exon 12, which was found only in affected family members. This G to A transition changes a highly conserved glutamic acid residue to a lysine residue in domain II S2 of the P/Q-type calcium channel α_{1A} ($Ca_v2.1\alpha_1$) subunit. Expression of the mutant channel *in vitro*, resulted in complete loss of channel function.

Clinical and Molecular Studies in 5 families with Limb Girdle Muscular Dystrophy/Hereditary Inclusion Body Myopathy, Paget disease of Bone and Frontotemporal dementia. *G.D.J. Watts¹, M. Thorne¹, M.J. Kovach², A. Pestronk³, V.E. Kimonis¹.* 1) Department of Genetics, Children's Hospital Boston, Harvard Medical School, Boston, MA; 2) Department of Biological and Environmental Sciences, University of Tennessee at Chattanooga, Chattanooga, TN; 3) Dept. of Neurology, Washington Univ. School of Med., St Louis, MO.

We have previously demonstrated linkage to 9p 13.3-p12 in a large family with Limb Girdle Muscular dystrophy and Paget disease of bone and an additional 3 families with autosomal dominant inclusion body myopathy (HIBM), Paget disease of bone (PDB) and frontotemporal dementia (Kovach et al 2001). Linkage in these families and an additional one with this unique combination identifies a critical locus on 9p 13.3-p12 spanning 4-6 MB.

Clinical features in 57 affected individuals in 5 families indicate that 51 had muscle weakness in variable patterns involving proximal, and, occasionally, distal muscles. With disease progression, the weakness became more generalized, often resulting in respiratory failure.

Muscle histology reveals myopathic changes and rimmed vacuoles. PDB caused by overactive osteoclasts was present in 51/57 primarily involving the spine and hip, causing pain, elevated alkaline phosphatase, and urine pyridinoline/ deoxypyridinoline and is responsive to bisphosphonates. Frontotemporal dementia associated with relative sparing of memory and impairment of executive skills occurred in 21/57 at a mean age of 54 y.

Recently mutations in the GNE (UDP-N-acetylglucosamine 2-epimerase) gene which maps to 9p13-p12 have been found in IBM2, an autosomal recessive inclusion body myopathy associated with quadriceps sparing. No mutations were found in the GNE gene in affected individuals in our families indicating that LGMD/HIBM/PDB/FTD is not allelic with IBM2. Candidate gene tropomyosin 2, NDUFB6 and SMU1 were also not implicated. Identification of the genes for this complex disorder is a priority in understanding these common pathways/ pathogenetic mechanisms.

Three-Year Experience with Mutation Studies for the International Long QT Syndrome Registry at Rochester: Screening, Molecular Spectrum, Mutation "Hot-spots", Phenotypic Variations, and Genotype-Phenotype

Correlation. *M. Qi*^{1,2}, *A. Moss*³, *L. Ning*^{1,2}, *W. Zareba*³, *J. Robinson*³, *Y. Xu*^{1,2}, *S. Rosero*³, *E.S. Kaufman*⁴, *C. Ohle*⁵, *T. Kelly*⁵, *D. Ryan*¹. 1) Dept Pathol/Lab Med; 2) Center for Cardiovascular Research; 3) Dept Med, Univ Rochester, Rochester, NY; 4) Heart & Vasc Res Ctr, MetroHealth Campus, Case Western Reserve Univ, Cleveland, OH; 5) Dept Ped (Genet), Univ Virginia, Charlottesville, VA.

Multiple cardiac ion channel genes have been identified in the hereditary long QT syndrome (LQTS), an arrhythmogenic disorder characterized by an unusual electrocardiographic abnormality (QT prolongation) and a propensity to syncope, polymorphous ventricular tachycardia (torsade de pointes), and sudden death. Since the establishment of the molecular genetics LQTS research program at URMC in 1999, we have set up molecular testing for all 6 known LQT genes and over 400 patients have been analyzed. Multiple novel mutations responsible for LQTS have been identified. Due to their multiple loci and considerable size, mutation detection in these genes represents a challenge that is only partially met by the conventional screening method of single stranded conformational polymorphism (SSCP). We tested the applicability of dHPLC in the molecular diagnosis of LQTS by assessing a cohort of 194 patients with 12 previously identified mutations (including 8 different missense mutations, 1-bp, 2-bp, 3-bp and 9-bp deletion mutations), and 2 polymorphisms in the LQTS genes. All mutations/SNPs were readily detected, including one undetectable by SSCP previously. Our data showed that the dHPLC technology is highly sensitive and efficient and it is now routinely used in our molecular analysis of LQTS. Multiple types of pathogenic mutations have been identified, including missense, nonsense, splice site, in-frame deletions, and frameshift mutations. While those mutations could be found in nearly all coding regions of the responsible genes, they were particularly frequent in the pore regions. The mutations in the HERG pore region manifest most severe clinical phenotypes for LQTS2. LQTS shows considerable variation in reduced penetrance and expressivity, even within families.

Pantothenate kinase associated neurodegeneration: *PANK2* gene structure, mutations, and molecular diagnosis.

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Pantothenate kinase associated neurodegeneration (PKAN), previously known as Hallervorden-Spatz syndrome, is an autosomal recessive neurodegenerative disorder caused by mutations in the pantothenate kinase 2 gene (*PANK2*). The *PANK2* sequence was obtained from two adjacent chromosome 20p clones. The mRNA is approximately 1.85 kb by Northern blot analysis. The cDNA is predicted to be 1383 nt from start to stop codon, encoding a protein of 460 amino acids with a predicted molecular weight of 50.6 kDa. Details on the structure of the gene were determined from RACE PCR and bioinformatics research. *PANK2* mRNA has 7 exons, initiated by several alternative first exons. Tissue expression analyzed by PCR from cDNA collections indicates a global expression pattern.

To date, 64 distinct *PANK2* mutations and 8 variations have been identified. All but 2 of 16 deletion or insertion mutations cause frameshifts resulting in a premature stop codon. These mutations, along with 7 nonsense and 5 splice site mutations, are predicted to result in no protein product. The G411R and T418M missense mutations in exon 6 are the most common mutations found in PKAN patients, together representing over 30% of mutant alleles. G411R occurs on a common haplotype of markers in 15 of 19 haplotyped patients. This suggests a founder effect about 400-1600 years ago. G411 is conserved among all human and mouse PANK paralogs and many pantothenate kinases from other species. Most other mutations in PKAN patients are present only in a single family. A screening protocol for genetic testing and prenatal diagnosis using PCR and sequencing has been developed. Information obtained from the study of *PANK2* mutations in PKAN patients may provide clues to the function, structure, catalysis, localization and intermolecular interactions of the PANK2 protein and the importance of specific amino acids or domains in fulfilling those roles.

Deletion analysis of the 15q11-q13 region by real-time quantitative PCR. *G. Raca*¹, *R. Brown*¹, *K. Buiting*², *S. Das*¹. 1) Department of Human Genetics, University of Chicago, Chicago, IL; 2) Institut für Humangenetik, Universitätsklinikum Essen, Essen, Germany.

Large gene deletions, particularly intragenic deletions, are often difficult to detect by standard PCR-based mutation detection methodologies. A promising approach to the identification of gene deletions is by real-time quantitative PCR (QPCR). We have applied this technique to the identification of deletions in the imprinting control center (IC) region on chromosome 15 in patients with Prader-Willi (PWS) and Angelman syndrome (AS), as a model for the study of gene and intragenic deletions by real-time QPCR. The PCR was performed in real-time using fluorescent hybridization probes for detection, in a duplex reaction with a control gene, using the LightCycler system. The *SNRPN* exon 1 and IC3 regions, which represent the smallest region of overlap for the PWS-IC and AS-IC regions respectively were the targeted regions for analysis and the b-globin gene was used as the control. We have analyzed over 50 normal DNA samples, 15 samples with known AS-IC deletions, and 15 samples with known PWS-IC deletions. For all the normal DNA samples the ratio of AS-IC or PWS-IC to b-globin was between 0.8 and 1.3. All samples with a deletion gave a ratio of 0.4 to 0.6, indicating a decrease in the relative concentration of the IC region as compared to the b-globin control. In addition, we have analyzed a series of 13 blinded samples and correctly identified the deletion status of all the samples. This assay will be important for the deletion analysis of the IC region in patients with PWS and AS and can be used for diagnostic purposes. We are currently performing a study to determine the incidence of intragenic deletions in the *UBE3A* gene in patients with AS. Mutations in the *UBE3A* gene have only been found in 14-38% of AS patients with normal methylation and it is possible that large intragenic deletions of the *UBE3A* gene are being missed in this group of patients. We are analyzing a series of patients with an AS phenotype and no identifiable molecular abnormality for deletions in the *UBE3A* gene by real-time QPCR. Results of our deletion analysis will be presented.

Ellis-van Creveld: A genetically heterogenous syndrome? *S.W.J. Tompson¹, V. Ruiz-Perez¹, E. Oliveira da Silva², L. Algazali³, M. Wright¹, J. Goodship¹*. 1) Institute of Human Genetics, University of Newcastle, Newcastle upon Tyne, UK; 2) Instituto Materno-infantil de Pernambuco, Recife, Brazil; 3) Faculty of Medicine, United Arab Emirates University, UAE.

Ellis-van Creveld syndrome [MIM 225500], an autosomal recessive skeletal dysplasia, is characterized by short limbs, short ribs, postaxial polydactyly, dysplastic nails and teeth and congenital cardiac defects. We have now screened 58 samples for mutations by direct sequencing of coding exons and fluorescent dosage PCR analysis. In total we have identified mutations in 13 patients (4 previously reported) which include deletions, insertions, missense, nonsense and splicing mutations. In all of these patients both changes have been identified and three mutations were seen in more than one family (S307P, 873insT, 1694delC). The lack of mutations in 45 patients (77%) raises the possibility of genetic heterogeneity. We have, therefore, carried out microsatellite analysis in 7 consanguineous pedigrees with no identified mutation. Affected individuals in each of these families were homozygous across the EvC gene and surrounding region. In a large consanguineous Brazilian family with six affecteds included in the analysis, an unaffected individual placed the mutation upstream of exon 12 and yet the causative mutation remains elusive. The mapping is therefore consistent with EvC being a single gene disorder; the fact that we have not identified mutations in the majority of patients suggests a common mutation mechanism that is missed by exon sequencing, such as an inversion. We obtained fibroblasts from two patients and the cDNA sequence was normal arguing against this hypothesis and again raises the possibility of genetic heterogeneity. This issue is yet to be resolved. We have also undertaken microsatellite analysis in consanguineous families with the phenotypically overlapping disorders, Jeunes asphyxiating thoracic dysplasia and short-rib polydactyly type III syndrome (SRPIII), and found that affected individuals are not homozygous for this region indicating that these disorders are not allelic with EvC.

Gross rearrangements in the *MECP2* gene in two patients with Rett syndrome. *E. Schollen, E. Deflem, E. Smeets, J.P. Fryns, G. Matthijs.* Center for Human Genetics, Univ Leuven, Leuven, Belgium.

Since the identification of *MECP2* as the disease gene in Rett syndrome in 1999 (MIM #312750), numerous publications describe mutations in this gene. Depending on the clinical selection criteria, mutations are found in 60-85% of the patients with typical Rett syndrome. The mutation spectrum in *MECP2* is mainly composed of single base changes, and small deletions in the C-terminal region. A limited number of more complicated rearrangements or bigger deletions (up to 400 bp) have been described. Most of the screenings are PCR based and restricted to the coding region. Therefore, major rearrangements or mutations in the regulatory regions might be missed.

Routine DHPLC screening of *MECP2* identified mutations in 43 of 68 Belgian patients with typical Rett syndrome. All frequent mutations were represented in this group as well as some C-terminal deletions and 4 new point-mutations. In a further attempt to find the molecular defect in a selection of 15 patients with a typical Rett presentation but no mutations upon routine screening, Southern blot was performed with a probe encompassing the coding region of exon 4 and part of the 3'UTR. Abnormal patterns were detected in two patients, suggesting major rearrangements of this region. These most probably result in disruption of the 3'UTR of the *MECP2* gene. Although the exact function of the long 3'UTR is not yet known, the strong conservation of this region between mouse and human as well as the tissue dependent expression of the two *MECP2* transcripts point to an important regulatory function. Further experiments are ongoing to identify the precise genetic defect.

These observations are unique but underline the limitations of the PCR-based diagnostic approaches.

MECP2 mutation screening in Brazilian patients with Angelman syndrome-like phenotype. *C. Fridman, M.C. Varela, C.P. Koiffmann.* Dept Biol, Univ Sao Paulo, Sao Paulo, Brazil. cintiafri@hotmail.com.

Angelman syndrome (AS) is characterized by hypotonia, severe mental retardation, absent speech, seizures, ataxia, outbursts of laughter, macrostomia and prognathism. Most of the cases present genetic abnormalities at chromosome 15q11-q13 (70% maternal deletion, 1-5% paternal uniparental disomy and ~8% UBE3A mutations), in ~10% of patients the genetic mechanism is unknown. Rett syndrome (RTT) is one of the main cause of severe mental retardation in girls and is characterized by autism, apraxia, seizures, stereotypic hand movements, and deceleration of head growth. After a period of normal development, the patients show a regression of motor and mental abilities (between 6 months and 3 years); they gradually lose speech and purposeful hand use. It is a dominant X-linked disorder, usually associated with male lethality, caused by mutations in the MECP2 gene. MECP2 mutations may occur in patients with typical and also atypical RTT phenotype showing that there is a broader spectrum of the disease. Since one of the differential diagnosis of AS is RTT, we have screened for mutations in the MECP2 gene 28 female patients with a clinical course compatible with AS in whom SNRPN methylation and UBE3A mutation analysis were normal. The main phenotypic and behavioral characteristics considered for AS inclusion criteria were mental retardation, neurodevelopmental delay, language impairment, happy demeanour and/or outbursts of laughter. PCR amplification was done with primers covering the MECP2 coding exons and the products were sequenced on an automated sequencer using the Dye Terminator method; the results were compared with the reference human MECP2 sequence (GenBankX99686). Two missense mutations (R306M, T158M) and two polymorphisms (S411) were identified. One of the polymorphisms was also present in the patient with T158M mutation. Since methylation analysis of SNRPN gene does not allow diagnosis in 10% of the AS patients, female patients with neurodevelopmental delay, mental retardation, language impairment, happy demeanour, inability to walk or ataxic gait and normal methylation pattern should be tested for MECP2 mutations. Support: FAPESP, CEPID, CNPq.

Identification of a novel mutation in *FBNI* in a patient with tall stature and ectopia lentis lacking marfanoid habitus. *M. Mamada, T. Yorifuji, K. Kurokawa, M. Kawai, T. Nakahata.* Pediatrics, Kyoto University Hospital, Kyoto, Japan.

Introduction: Marfan syndrome (MFS) is an autosomal dominant heritable disorder of connective tissue. The defective protein is fibrillin-1, the 350-kDa extracellular glycoprotein, encoded by *FBNI* mapped to chromosome 15q21.1. Mutations in *FBNI* give rise to not only MFS but also other related disorders such as isolated ectopia lentis, Shprintzen-Goldberg syndrome and MASS phenotype. We report a case characterized by tall stature and ectopia lentis with a defect in *FBNI*. **Patient Profile:** Patient is a 9-year-old boy who was born to clinically normal parents. Bilateral ectopia lentis was diagnosed at the age of 3 years. He has no cardiac manifestations other than annuloaortic ectasia. As skeletal features he has only tall stature (3.5SD), completely lacking marfanoid habitus and other skeletal anomalies. He does not fulfil the Gent criteria for MFS. **Materials and Methods:** Mutation in the *FBNI* mRNA was detected by RT-PCR amplification and direct sequence analysis. Southern blot analysis was carried out to confirm the mutation in the genomic DNA. **Results:** Direct sequence analysis revealed the 459 bp in-frame deletion covering a part of exon 6, whole of exons 7 and 8 and a part of exon 9. What was a distinctive feature was the same 12 bp sequence appeared at both ends of the deletion. Southern blot analysis did not reveal any difference of the band pattern compared to the control, showing the deletion was existed only in patients mRNA, not in genomic DNA. No sequence alteration was detected in exons 6 to 9, suggesting that the splicing error did not explain the mechanism of this mutation. **Discussion:** In this study we identified a novel mutation in *FBNI* with a patient of unique phenotype that has not been reported to date. The phenotype is very interesting when we consider the genetic factors to affect stature, because the defect of *FBNI* is associated with tall stature showing no typical skeletal features of MFS. This suggests that tall stature and Marfan-like skeletal form can be separated in the fibrillinopathy. To our knowledge this type of mutation has not been reported to date. Its mechanism gives us a great interest and is now elucidating.

Haplotype analysis of the tyrosinase (*TYR*) gene. *R.A. King, J.E. Pietsch, J.D. Lande, J.P. Fryer, W.S. Oetting.*
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Tyrosinase is the major enzyme involved in pigmentation in all animal species, and the human *TYR* gene and its protein product show great homology to lower organisms. Mutations of the *TYR* gene produce oculocutaneous albinism type 1 (OCA1) and more than 100 different mutations in OCA1 have been identified. We have now created extended haplotypes of the *TYR* gene to analyze the evolution of the human gene and the origin of the mutations. Four single nucleotide polymorphisms (SNPs) were identified in the 5' region of the *TYR* gene by sequencing 4000 bp of the 5' promoter region in DNA from 13 families [-3879 (g->a), -3748 (a->c), -3421 (g->a), and 3046 (g->a)]. A polymorphic LINE insertion (at 2093) was also identified. Extended haplotypes were then constructed from analysis of 159 chromosomes from probands and parents of 54 OCA families, and 151 chromosomes from 59 control normally-pigmented trios (child and parents). All samples were predominantly Caucasian. The extended haplotypes included the 4 new SNPs, the insertion, and the known polymorphisms at 301 (c->t), -199 (c->a), codon 192 (C->A), codon 402 (G->A), and the *Taq* I site in intron 1. The chimpanzee *TYR* gene was used to determine the root ancestral haplotype. A total of 21 extended haplotypes were identified in 310 chromosomes. The haplotype distribution of 31 different mutations associated with OCA1 showed that all but one mutation occurred on a single haplotype indicating that most were founder and not recurrent mutations; one mutation was found on 2 distinct haplotypes. Components of the Phylip analysis package were used to construct the most parsimonious phylogenetic haplotype tree, showing three major evolutionary branches in the development of this gene. 21/31 (68%) of the mutations were on 3 haplotypes. We conclude that tyrosinase gene haplotypes associated with OCA1 mutations provide a historical view of this gene in humans.

Analysis of CFTR gene mutation frequency in a random European population. *G. Modiano*¹, *C. Bombieri*², *M. des Georges*³, *V. Scotet*⁴, *M. Toepfer*⁵, *B.M. Ciminelli*¹, *F. Belpinati*², *C. Guittard*³, *M.P. Audrezet*⁴, *S. Giorgi*¹, *A. Begnini*², *M. Koudova*⁵, *F. Pompei*¹, *C. Ciccacci*¹, *M. Macek jr*⁵, *FC. Ferec*⁴, *M. Claustres*³, *P.F. Pignatti*². 1) Dpt. Biology E Calef, Univ. Rome Tor Vergata, Italy; 2) Sec. Biol. Genet, DMIBG, Univ. Verona, Italy; 3) Inst. Biol., Univ. Montpellier, France; 4) Centre de Biogenetique CDTS, Brest, France; 5) Dpt. of Mol. Genet. CF-Centre Charles Univ., Prague, Czech Rep.

In a study on 2530 CFTR alleles from random individuals from Italy, France, and Czech Republic, we have identified 16 CFTR Single Nucleotide Substitutions (10 of which in the coding sequence: cSNS) which are certainly non CF-causing because for each of them the frequency ($q - 2.5$ se) is higher than the combined frequency for Europe of the non well characterized CF-causing alleles (ca. 0.004). The sample size required for this approach has been so large as to make possible to study the pattern of variation of the CFTR genes carrying the M470 allele (the ancestral one) separately from those with the 470V allele. It turned out that all types of variation are preferentially located in the M470-CFTR genes; indeed some of them are even 'M470 allele-restricted'. Due to this heterogeneous distribution of the CFTR gene variability among its two M470V alleles it is possible to estimate from the present data that the probability of being a CF risk couple for the MM x MM couples is about 130 fold higher than for the VV x VV couples. The present study has been concerned with the coding sequence of only one gene. However, the sample size was so large as to allow one to examine a range of variability (the sub-polymorphic quasi-polymorphic range) never explored before. The main findings of this large scale study have been the following: 1) the number of detected cSNS is proportional to the square root of n genomes; 2) the cSNS-MS (MisSense) have a probability of occurring with a sub-Polymorphic frequency equal to that of the cSNS-SS (SameSense), whereas the probability of the cSNS-MS of being polymorphic is about fourfold lower than that of the cSNS-SS. This implies that selection has prevented the vast majority of the cSNS-MS from becoming polymorphic.

Evidence of a founder effect for the RETGC1 (GUCY2D) 2943delG mutation in Leber congenital amaurosis pedigrees of finnish origin. *S. Hanein¹, I. Perrault¹, P. Olsen², T. Lopponen², M. Hietala³, S. Gerber¹, M. Jeanpierre⁴, F. Barbet¹, D. Ducroq¹, S. Hakiki¹, A. Munnich¹, J.M. Rozet¹, J. Kaplan¹.* 1) INSERM U393, Hopital des Enfants Malades, Paris CDX15, FRANCE; 2) Dpt of Clinical Genetics, Oulu University Hospital, Oulu, Finland; 3) Clinical Genetics Unit, Turku University Hospital, Turku, Finland; 4) INSERM-EMI 0005, Hopital Cochin, Paris CDX14, France.

Leber congenital amaurosis (LCA) is the earliest and most severe form of all inherited retinal dystrophies. It is a genetically heterogeneous condition as six disease-causing genes have been hitherto identified. RETGC1 (GUCY2D) is more frequently implicated in our series of LCA patients. Interestingly, 70 % of the families with RETGC1 mutations are originating from Mediterranean countries, the remaining families (30 %) originating from various regions worldwide. Here, we report the screening of the RETGC1 gene in three unrelated and non consanguineous families of Finnish origin. The 21 exons of the RETGC1 gene as well as intron-exon boundaries were screened for mutations in one affected patient of the three families using DHPLC and direct sequencing. Linkage disequilibrium studies were performed by haplotyping all available family members of the three using polymorphic markers flanking the RETGC1 gene. All patients of the three Finnish families studied here were found to carry the same homozygous delG2943 mutation. The segregation of the mutation with the disease was confirmed in all three pedigrees. The identification of a unique homozygous mutation in three unrelated and non consanguineous families suggests a founder effect. Interestingly, no linkage disequilibrium was found using polymorphic markers flanking the RETGC1 gene, supporting the view that the mutation was very ancient. Bayesian calculations points the founder mutation to 150 generations (95% credible interval 80-240 generations), ie 3000 years ago.

Survey of Molecular Lesions Causing Genetic Disease in the Old Order Amish and Conservative Mennonites of Lancaster County, PA. *E.G. Puffenberger, D.H. Morton.* Clinic for Special Children, Strasburg, PA.

The Clinic for Special Children is a non-profit medical service for patients with inherited metabolic disorders. The clinic mainly serves Old Order Amish and conservative Mennonite families in eastern Pennsylvania. These Plain sects have an unusually high incidence of specific genetic diseases. In a continuing effort to provide molecular diagnostic services, we have identified and/or verified the molecular lesions which segregate in these populations. We and others have identified 34 molecular lesions in 26 different genes which cause disease in these two groups. For each disease, we have verified the published mutations in our patient population. For diseases where the mutation had not been previously identified, we sequenced the genes and identified mutations. In our survey, 20 disorders were caused by a single mutation segregating in the population. However, 6 monogenic diseases exhibited mutation heterogeneity. Notably, we sequenced the SLC3A1 and SLC7A9 genes in five Mennonite cystinuria patients. We were surprised to identify 4 separate mutations (2 at each locus), 3 of which were novel. Other disorders demonstrating mutation heterogeneity include congenital nephrotic syndrome, mevalonate kinase deficiency, phenylketonuria, and familial hypercholanemia. Several diseases are found in both populations. This is not wholly unexpected as there are known genealogical ties and they have a shared Anabaptist history. In Crigler-Najjar syndrome, the same mutation in UGT1A1 segregates in both populations. We confirmed a common haplotype by microsatellite marker analysis of the UGT1A1 region on chromosome 2. Conversely, 3-methylcrotonylglycinuria is found in both populations, but a different mutation in MCCB causes disease. Assessment of mutation frequency was performed for several diseases using disease incidence and genotyping. For example, we estimated the incidence of maple syrup urine disease (MSUD) in the Mennonites for the period 1985-1994. The incidence, 1/378 births, yielded a carrier frequency of 9.8% (without correction for inbreeding) and a carrier frequency of 7.7% with an inbreeding coefficient of 0.0219.

The dominant-negative action of mutant SOX10 seen in patients with simple WS4 is potentially diminished apparently due to nonsense-mediated decay. *M. Khajavi, K. Inoue, J.R. Lupski.* Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.

Waardenburg's syndrome (WS) is an autosomal dominant disorder which causes deafness and pigmentary disturbances due to defective function of the embryonic neural crest. In association with sensorineural deafness and pigmentary abnormalities, congenital aganglionosis has been reported, a combination called Waardenburg-Hirschsprung syndrome type 4 (WS4). The involvement of two neural crest derived lineages indicates a common regulatory pathway shared between enteric ganglia and melanocytes. In the heterozygous state, spontaneous mutation of *SOX10* which results in premature terminations has been identified in patients afflicted with WS4. Each of these mutations is likely to result in a null allele, suggesting haploinsufficiency of the *SOX10* product. Interestingly, in a more severe neurocristopathy, including leukodystrophy compatible with Pelizaeus-Merzbacher disease (PMD) and CMT1-type peripheral myelinopathy accompanying WS4, were reported in which an extended proline rich tail that was added to the transactivation domain of the *SOX10* protein was observed. This led to the hypothesis that this mutant may give an additional or different function to the transactivation domain of *SOX10* and result in a dominant negative allele. Further mutations of *SOX10*, each of which results in premature terminations in the last exon of *SOX10*, have been reported in WS4 patients with both CNS and PNS myelinopathy. We hypothesized that the dominant-negative action of *SOX10* nonsense mutations in patients with simple WS4 is mitigated by the nonsense-mediated decay pathway. To address this hypothesis, we have characterized *SOX10* mutations found in WS4 patients with or without myelinopathies by introducing such disease associated mutations in an expression vector and using transiently transfected U138/cos7 cell lines. Both mutation types interfere with the wild-type *SOX10* transcription activity, suggesting dominant-negative functions. Current experiments are addressing a potential role for nonsense-mediated decay in the abrogation of dominant-negative effects of selected mutant alleles.

Non-classic cystic fibrosis phenotypes in the absence of *CFTR* mutations. *J.D. Groman*^{1,2}, *B. Karczeski*¹, *M.E. Meyer*¹, *P.L. Zeitlin*³, *R.W. Wilmott*⁴, *G.R. Cutting*^{1,2,3}. 1) McKusick-Nathans Inst. of Gen. Med., Johns Hopkins Sch. of Med., Baltimore, MD; 2) Training Program in Human Genetics, Johns Hopkins University Sch. of Med., Baltimore, MD; 3) Dept. of Pediatrics, Johns Hopkins Sch. of Med., Baltimore, MD; 4) Dept. of Pediatrics, Saint Louis Univ. Sch. of Med., St. Louis, MO.

Identification of *CFTR*, the gene responsible for Cystic Fibrosis, is regarded as one of the triumphs of positional cloning. Subsequently, mutations causing loss of function were identified in each *CFTR* gene in patients with classic CF, while mutations that reduce but do not eliminate *CFTR* function were found in patients with non-classic forms of CF. Patients with the classic form of CF have manifestations in the sweat gland, pancreas, respiratory tract, and male reproductive tract, whereas patients with non-classic CF have manifestations limited to a subset of the aforementioned organ systems. To determine the entire spectrum of mutations associated with non-classic CF phenotypes, we performed extensive analysis of the *CFTR* gene on 99 non-classic CF patients who failed to test positive for two CF causing mutations prior to referral. Mutations in each *CFTR* gene were identified in 35 of 49 patients referred with one previously identified mutation, but in only 6 of 50 patients referred without a mutation. Twenty-three patients had one, and 35 patients had no *CFTR* mutations. Haplotype analysis revealed lack of linkage to *CFTR* in two unrelated families with affected siblings. Although each of the affected siblings had elevated pilocarpine induced sweat Cl⁻ concentrations, measurements of cAMP-mediated ion and fluid transport demonstrated the presence of functional *CFTR*. No significant clinical differences were found among patients with or without *CFTR* mutations, except pancreatic insufficiency was less common in patients with two *CFTR* mutations (5%) compared to those with one (22%), or without *CFTR* mutations (31%; $p = 0.006$). These findings suggest that factors other than mutations in the *CFTR* gene can produce phenotypes clinically indistinguishable from CF caused by *CFTR* dysfunction.

Trans-splicing of mRNA precursor results in duplication or triplication of exon 2 in dystrophin mRNA and is responsible for Duchenne muscular dystrophy. *Y. Takeshima*¹, *T. Ito*², *A. Surono*², *H. Nakamura*¹, *M. Matsuo*². 1) Department of Pediatrics, Kobe Univ. Grad. Sch. Med, Kobe, Japan; 2) Division of Molecular Medicine, Kobe Univ. Grad. Sch. Med, Kobe, Japan.

Splicing is divided into two patterns; one is cis-splicing, where two exons located same pre-mRNA, and the other is trans-splicing, where the two exons are initially parts of two separate pre-mRNA molecules. The dystrophin gene, which is mutated in Duchenne muscular dystrophy (DMD), is the largest human gene and consists of 79 constitutive exons and two cryptic exons X and 2a located in introns 1 and 2, respectively. In the present study, we isolated several dystrophin cDNAs with different 5'-terminal structure which were caused by trans-splicing in Japanese DMD case.

When a region encompassing exons 1 to 5 was amplified from dystrophin cDNA in his lymphocytes, only one cDNA consisting of exons 1-X-2-2-3 was identified. From his skeletal muscle, remarkably several different dystrophin cDNAs were isolated in different amount. Two major cDNAs consists of exons 1-2-2-3 or 1-X-2-2-3, and the others consists of exons 1-2-X-2-2-3, 1-2-2a-3, 1-X-2-3, 1-2-3. Since these cDNAs suggested duplication or triplication of exon 2 in his genomic DNA, genomic DNA was examined by Southern blot analysis and PCR amplification. Unexpectedly it was disclosed that only single copy of exon 2 was present and nucleotide sequence of exon 2 and exon/intron boundary is normal.

These results indicate that duplication or triplication of exon 2 in mRNA of index case is due to the trans-splicing between different pre-mRNA molecules of dystrophin. And this is a novel molecular mechanism of human genetic disorders.

Identification of Novel Mutations in Two Amelogenesis Imperfecta Families. *P.S. Hart¹, T.C. Hart², K.W. Seow³, A.J. Aldred⁴, M.D. Michalec¹, J.T. Wright⁵*. 1) Dept Human Gen, Univ Pittsburgh, Pittsburgh, PA; 2) Dept Oral Med/Pathology, Univ Pittsburgh, Pittsburgh, PA; 3) Dept Ped Dent, Univ Queensland, Queensland, Australia; 4) Dept Dent and Murdoch Children's Research Institute, Royal Children's Hospital, Melbourne, Australia; 5) Dept Ped Dent, Univ North Carolina, Chapel Hill, NC.

The Amelogenesis Imperfectas (AI) are inherited disorders that adversely affect enamel development causing abnormalities in amount, structure and composition of enamel. The genetic basis for three forms of AI are known. The X-linked form, AIH1, results from amelogenin gene (AMELX) mutations. 2 clinically distinct forms of autosomal dominant AI, smooth hypoplastic AI and local hypoplastic AI, are due to enamelin gene mutations (ENAM) on 4q21. We present mutational analyses of 2 families with AI: one with the X-linked and one with an autosomal dominant form. The X-linked form is due to an AMELX exon 2 C to G transversion (g.52C>T; c.52C>T). This mutation changes a highly conserved proline to an alanine (p.P18A), and heterozygous females exhibited vertical grooves of hypoplastic enamel. This brings the number of described amelogenin gene mutations to 13, with 54% being deletions, 31% missense mutations and 15% nonsense mutations. The family segregating autosomal dominant AI presented a smooth hypoplastic AI phenotype. Enamelin gene analysis revealed a deletion of a G in exon 8 (c.588delG). This frameshift creates a smaller (276 amino acid) chimeric protein with 80 novel amino acids before premature termination (p.197fsX277), compared to the wild-type 1142 amino acids protein. This is the third mutation described in ENAM. The previously reported c.157A>T (p.K53X) mutation results in a localized hypoplastic phenotype. The c.534+1G>A (p.A158_178Qdel) mutation results in a more severe smooth hypoplastic phenotype. The more severe hypoplastic phenotype with the p.197fsX277 mutation described here may indicate a dominant negative effect of the mutant protein interfering with function of the wild-type protein. Mutational analysis of AI families provides insight into normal enamel development as well as demonstrating genotype-phenotype correlations.

Spinocerebellar ataxia type 10 ATTCT repeat expansions in Brazilian patients and in a patient with early onset ataxia. P. Fang¹, T. Matsuura², H.A.G. Teive³, S. Raskin⁴, P. Jayakar⁵, E. Schmitt¹, T. Ashizawa², B.B. Roa¹. 1) Dept Molec Human Genetics, Baylor Col Medicine, Houston, TX; 2) Dept Neurology, Baylor Col Medicine, Houston, TX; 3) Dept Medicine, Federal Univ Parana, Brazil; 4) Laboratorio Genetika, Brazil; 5) Div of Genetics and Metabolism, Miami Childrens Hospital, Miami, FL.

Spinocerebellar ataxia type 10 (SCA10) is an autosomal dominant neurogenetic disorder characterized by cerebellar ataxia and seizures. SCA10 is associated with a large ATTCT pentanucleotide repeat expansion mutation in the *SCA10* gene. Normal individuals have 10-22 repeats, while SCA10 patients have expansions in the hundreds to thousands of repeats. We report on DNA diagnostic testing for three SCA10 patients. Two patients were from unrelated Brazilian families of Portuguese ancestry who were referred for SCA10 diagnostic testing because of progressive gait ataxia associated with dysarthria; previous molecular testing for SCA1, 2, 3, 6, 7 and 8 were negative. Both patients had one normal allele by PCR analysis of the polymorphic ATTCT repeat region of *SCA10*. Southern analysis found expansions of ~1,500 repeats for patient 1, and ~1,600 repeats for patient 2. The third patient is a 19-year old Hispanic female from the U.S. Southern analysis found an expansion of ~280 repeats in the proband and in her apparently asymptomatic mother, but not in her asymptomatic father. Southern data were corroborated by a modified PCR method which confirmed ATTCT repeat expansions in the two Brazilian patients, the 19-year old patient and her mother. Previous studies had documented SCA10 expansion mutations exclusively in the Mexican population. The incidence for other populations is believed to be low, but is currently unknown. Our study of the Brazilian patients presents the first SCA10 expansion cases identified in non-Mexican ataxia patients, putting Portuguese/Brazilian populations at-risk for SCA10. The ~280-repeat expansion identified in the 19-year old patient represents the smallest SCA10 expansion mutation identified to date. The findings on this family highlight the need for further genotype-phenotype correlation studies for SCA10.

Characterization of mutant TATA-box binding protein (TBP) containing expanded polyglutamine tracts. *M.J. Friedman*^{1,3}, *K.J. Dougherty*², *S.H. Li*³, *X.J. Li*³. 1) Graduate Program in Genetics and Molecular Biology; 2) Graduate Program in Neuroscience; 3) Dept Human Genetics, Whitehead, Emory Univ, Atlanta, GA., USA.

Nine inherited neurodegenerative diseases have been found to associate with polyglutamine expansion in the respective disease proteins including TBP, the TATA binding protein that is essential for the basal transcription of genes. Expanded polyglutamine in TBP causes a neurodegenerative disorder (SCA17) with symptoms similar to Huntington's disease. Since TBP is a well-characterized protein, study of how polyglutamine expansion affects the function of TBP would provide insight into the mechanism(s) by which polyglutamine expansion mediates selective neurodegeneration. We have generated cDNA constructs by replacing the normal CAG repeat with expanded CAA/CAG repeats in the TBP gene. The resulting DNA constructs encode TBP containing 15, 38, 60, and 98 glutamines, and thus cover both the normal (15-40 glutamines) and pathogenic (42-67 glutamines) ranges observed in humans. Expression of these cDNAs in HEK 293 cells demonstrated that only pathogenic repeats caused TBP to form aggregates. While these aggregates were unable to react with 1C2 antibody that was generated against TBP, western blots showed that 1C2 preferentially reacted with soluble TBP containing expanded polyglutamine, suggesting that polyglutamine expansion confers different protein conformation and immunoreactivity. HEK 293 cells expressing mutant TBP also had reduced viability as compared with those expressing normal TBP. The TBP disease cell model will allow us to study further how polyglutamine expansion alters protein conformation and the function of TBP.

Mutant huntingtin forms aggregates differently in the nucleus and neuropil. *J.R. McGuire^{1,2}, Z.X. Yu², H. Zhou², S.H. Li², X.J. Li².* 1) Graduate Program in Genetics and Molecular Biology, Emory Univ, Atlanta, GA; 2) Dept Human Genetics, Emory Univ, Atlanta, GA.

Huntington disease is caused by polyglutamine expansion in the disease protein, huntingtin, a 350 kDa protein of unknown function. Expanded polyglutamine causes huntingtin to kill neurons and forms aggregates in the nucleus. While the role of intranuclear aggregates is unclear, our recent studies suggest that the formation of neuropil aggregates is more correlated with disease progression in HD mice and that neuropil aggregates are associated with axonal degeneration. To examine whether these two types of aggregates are formed differently, we examined HD repeat knock-in mice that express full-length mutant huntingtin and show both intranuclear and neuropil aggregates. We first used the antibody EM48 that reacts with the first 253 amino acids of huntingtin. EM48 recognizes aggregates in both the nucleus and neuropil. Our newly developed antibody EM121, which reacts with amino acids 359-429 of huntingtin, was unable to react with nuclear huntingtin aggregates despite its strong reaction with diffuse cytoplasmic huntingtin. PC12 cells were then transfected with N-terminal huntingtin (1-505 amino acids) that contains the HA epitope at its C-terminus. Mutant huntingtin containing a 120 glutamine repeat formed aggregates in the cytoplasm and neuritis, which could be labeled by both EM48 and the anti-HA antibody. Some transfected mutant huntingtin also formed intranuclear inclusions that were intensively labeled by EM48 but not anti-HA. In HD repeat knock-in mice, nuclear huntingtin aggregates were ubiquitinated while neuropil aggregates were negative to anti-ubiquitin. These studies suggest that intranuclear aggregates are formed by huntingtin fragments smaller than those for neuropil aggregates and therefore are more readily ubiquitinated.

Implementation of Long PCR for the Detection of a Very Large Trinucleotide Repeat Expansion in a 3-Year-old with Juvenile Huntington's Disease. *T.A. Maher¹, M. Ito¹, B.A. Loose¹, B.T. Darras², J.M. Milunsky^{1,3}*. 1) Center for Human Genetics, Boston University School of Medicine, Boston, MA; 2) Department of Neurology, Children's Hospital, Harvard Medical School, Boston, MA; 3) Department of Pediatrics, Boston University School of Medicine, Boston, MA.

A 3-year-old female with juvenile Huntington's disease (HD) and a family history is described. To our knowledge, she is one of the youngest children reported with juvenile HD and molecular confirmation. Symptoms of developmental regression, seizures and rigidity were noted at 18 months. HD, a progressive disorder of motor, cognitive, and psychiatric disturbances, is inherited in an autosomal dominant manner with paternal anticipation. The prevalence is between 3 and 7 per 100,000 in populations of western European descent. Juvenile HD has onset of symptoms before age 20 and is documented in less than 10% of all cases. The diagnosis of HD rests on family history, characteristic clinical findings, and the detection of an expansion of a CAG polyglutamine tract in the Huntingtin gene on chromosome 4p16. The CAG repeat length in affected individuals ranges from 36 to more than 200. The expansion of the CAG triplet repeat in the IT-15 (Huntingtin) gene of this patient was determined by a modified PCR analysis to be approximately 265 repeats, the largest CAG repeat ever reported in a HD allele. Currently, many molecular diagnostic laboratories in the US offer HD testing using a widely available PCR-based molecular test that is 98.8% sensitive, however, often the largest repeats are missed because of inefficient amplification of the large CAG repeat expansion. The Southern blot method is more reliable for such cases, however, only a few laboratories in the US have adopted this method. We have developed an easy, rapid and reliable modified PCR method using XL PCR that can detect up to at least 265 CAG repeats. This assay was shown to be reproducible, very reliable, and this report suggests implementation of this assay procedure as part of a standard protocol for HD testing in the molecular diagnostic community.

Huntington's disease-like 2 (HDL2): Frequency, genotype-phenotype relationships, and neuropathological characterization. *R.L. Margolis^{1,2}, J. Troncoso³, S.E. Holmes¹, E. O'Hearn^{4,5}, A. Rosenblatt¹, C. Callahan¹, J. Hwang¹, D. Rodnicki¹, C.A. Ross^{1,2,5}, A. Krause⁶, W. Seltzer⁷.* 1) Dept of Psychiatry; 2) Program in Cellular and Molecular Medicine; 3) Dept of Pathology; 4) Dept of Neurology; 5) Dept of Neuroscience, Johns Hopkins Univ Sch Med, Baltimore, MD; 6) Div of Human Genetics, National Health Laboratory Service, and Sch of Pathology, Univ of the Witwatersrand, Johannesburg, S. Africa; 7) Athena Diagnostics, Inc., Worcester, MA.

We recently reported that a CAG/CTG expansion in a variably spliced exon of Junctophilin-3 on 16q causes a neuropsychiatric disorder, termed Huntington's disease-like 2 (HDL2), that is clinically indistinguishable from Huntington's disease (HD). We have now confirmed the existence of this new disorder in at least seven independent pedigrees. Across all populations so far examined, HDL2 accounts for about 0.5-1% of HD-like cases that test negative for the HD gene, though the frequency of HDL2 is higher if cases are limited to those with an autosomal dominant inheritance and a phenotype typical of HD. Most pedigrees are of African descent, and a particularly high number of cases have been ascertained in S. Africa. Repeat length is tightly correlated with younger age of onset. Individuals with shorter expanded repeats (about 42-49 triplets) tend to present with prominent chorea, while those with longer expansions (50 or more triplets) tend to have more rigidity and dystonia (similar to juvenile onset HD). Almost all affected individuals have psychiatric symptoms or syndromes. The two brains available for autopsy both show an HD pattern of neurodegeneration and intranuclear inclusion bodies similar to those seen in HD.

CAG/CTG expansions are rare among patients with familial SCA not assigned to the SCA1-3,6-8,10,12,17 loci. A. Saluto¹, C. Cagnoli¹, C. Michielotto¹, C. Gellera², A. Castucci², C. Mariotti², S. DiDonato², L. Orsi³, A. Franco³, E. Rota³, F. Taroni², N. Migone¹, A. Brusco¹. 1) Genet.Biol.Bioch.& U.O.Genet Med,Osp.G.Battista,Torino,Italy; 2) Dip.Biochem.&Genetics-Ist.C.Besta,Milano,Italy; 3) Dip Neurosc.,Torino,Italy.

A CAG/CTG repeat expansion has been involved in 8 of the 9 known spinocerebellar ataxia (SCA) genes. We investigated the contribution of this expansion in orphan SCA genes, by using the RED technique. We selected 139 unrelated familial SCA patients of Italian origin, negative for expansions at the SCA1-3,6-8,10,12 and 17 genes: 52 were autosomal dominant and 87 with an uncertain transmission. Patients carrying 40 or less CAG/CTG were not considered for further analyses, since this size is present also among healthy controls. Twenty-four had an expansion of 50 or more triplets, and were screened for two polymorphic regions containing CAG/CTG, SEF2-1B and ERDA1 loci, not involved in the SCA pathogenesis. In 19 cases the expansion was attributed to the ERDA1, and in two to the SEF2-1B locus. Among the remaining cases, one (130 repeats) revealed to be an SCA8 case, escaped to the previous screening. The remaining two were evaluated for the DRPLA expansion, and indeed, one had a value in the full pathological range (63 triplets). The last patient was at the end suspected to be compatible with Huntington's disease (HD): in fact an expansion of 57 repeats was detected in the corresponding gene. Our screening on a large group of 139 familial SCA patients let us to conclude that orphan SCA genes have probably a pathogenic mechanism different from the CAG/CTG expansions. Alternatively we could hypothesize the existence of genes like TBP, where a mix of CAG/CAA triplets, which cannot be detected by RED, codes a pathogenic stretch of polyglutamines, or the presence of pathogenic ranges below 40 repeats. The involvement of repeat expansions in at least a part of these patients cannot be ruled out, also considering the presence of anticipation in at least ~10% of our families. Furthermore, a few cases diagnosed as SCA are probably due to mutations in the DRPLA, and eventually the HD gene.

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Huntington's disease-like 2 (HDL2): Preliminary exploration of pathogenesis. *D. Rudnicki¹, E. O'Hearn^{2,3}, S.E. Holmes¹, A. Sawa^{1,3}, Z. Kaminsky¹, J. Kleiderlein¹, J. Troncoso⁴, C.A. Ross^{1,3,5}, H. Takeshima⁶, T.H. Moran¹, R.L. Margolis^{1,5}.* 1) Department of Psychiatry; 2) Neurology; 3) Neuroscience; 4) Pathology and; 5) Program in Cellular and Molecular Medicine, Johns Hopkins University School of Medicine, Baltimore, MD; 6) Department of Biochemistry, Tohoku University, Japan.

Last year our laboratory identified a disorder, termed Huntington's Disease-Like 2 (HDL2; Margolis et al., 2001, Holmes et al., 2001), with clinical and pathological findings essentially indistinguishable from Huntington's disease (HD). The mutation causing HDL2 is a CAG/CTG expansion in the CTG orientation within a variably spliced exon of junctophilin-3 (JPH3). The position of this mutation suggests several possible hypotheses for HDL2 pathogenesis, including 1) the presence of a cryptic CAG repeat encoding glutamine on the reverse strand; 2) haploinsufficiency of the JPH3 gene resulting from interference of transcription, splicing, or translation by an expanded repeat; and 3) toxicity of an expanded polyalanine or polyleucine repeat encoded by a splice variant of JPH3. Using RT-PCR of normal and HDL2 brain tissue, we have yet to find evidence of the first hypothesis. To test the second hypothesis, haploinsufficiency of JPH3, we are examining the behavior and neuropathology of junctophilin-3 knockout mice (Nishi et al, 2002). Both junctophilin-3 *wt/-* and *-/-* mice show impaired motor function on an accelerating rotarod protocol as early as 6 weeks of age, with increasing deficits with advancing age. Performance was more impaired in the *-/-* mice. We are currently using immunohistochemistry to compare the brain of *wt/-* and *-/-* mouse brains to human HDL2 brain samples, and to search for evidence of polyleucine or polyalanine toxicity (hypothesis 3) in cell models and human HDL2 brain samples. Confirming one (or more) of these hypotheses may provide valuable insight into the mechanisms of striatal vulnerability and the pathogenesis of HDL2, HD and other neurodegenerative disorders. Funding sources: NINDS and the Hereditary Disease Foundation.

Exclusion of maternal uniparental disomy of chromosome 14 in Prader-Willi syndrome referrals using a rapid methylation PCR assay. *L. Dietz*¹, *A.A. Wylie*², *K.A. Rauen*³, *S.K. Murphy*², *R.L. Jirtle*³, *P.D. Cotter*^{1,3}. 1) Medical Genetics, Children's Hospital, Oakland, CA; 2) Department of Radiation Oncology, Duke University Medical Center, Durham, NC; 3) Division of Medical Genetics, Department of Pediatrics, University of California San Francisco, San Francisco, CA.

Maternal and paternal uniparental disomy of chromosome 14 are both associated with abnormal phenotypes. PatUPD14 is characterized by polyhydramnios, low birth weight and skeletal and abdominal anomalies. MatUPD14 has a less severe phenotype including growth retardation, short stature, precocious puberty, obesity, hypotonia and some developmental delay. To date, over twenty matUPD14 patients have been reported. Interestingly, there are some phenotypic features of matUPD14, notably hypotonia, obesity and hypogonadism in some patients, which are suggestive of Prader-Willi syndrome (PWS). Recently, three matUPD14 patients were reported who were originally referred for a possible diagnosis of PWS. The identification of matUPD14 in these patients suggested that there may be some utility in testing for matUPD14 in patients referred for PWS, who were not confirmed by molecular analysis. In order to test this hypothesis, we selected 200 patients who had been referred for molecular diagnosis of Prader-Willi syndrome based on their clinical phenotype, and who were normal by Southern blot or methylation PCR (mPCR) analysis of the SNRPN promoter region. Patients were screened using a rapid bisulfite modification/mPCR method based upon the differential methylation of the imprinted *GTL2/MEG3* gene on chromosome 14. Methylation PCR analysis of normal, patUPD14 and matUPD14 controls all showed the expected PCR fragments. All 200 patient samples showed both the paternal and maternal-specific PCR fragments, consistent with biparental inheritance of chromosome 14 and excluding matUPD14. This data suggests that the incidence of matUPD14 among patients referred for PWS is likely to be low.

Genetic Heterogeneity of Autosomal Recessive Cataract. *G.D. Billingsley¹, R. Sidhu¹, S. Herd¹, A.V. Levin³, F.L. Munier^{4,5}, D.F. Schorderet⁴, A. Balmer⁵, D.A. Mackey^{6,7}, M. Sale⁶, E. Héon^{1,2,3}.* 1) Vision Science Research Program, Toronto Western Hospital; 2) University of Toronto, Department of Ophthalmology; 3) The Hospital for Sick Children Research Institute; Toronto, Ontario, Canada; 4) Unit of Oculogenetics and Division of Medical Genetics, CHUV; 5) Hôpital Ophthalmique Jules Gonin, Lausanne, Switzerland; 6) Menzies Centre for Population Health Research, University of Tasmania, Hobart, Tasmania, Australia; 7) Centre for Eye Research Australia, University of Melbourne, East Melbourne, Victoria, Australia.

Cataracts constitute the 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, predominantly autosomal recessive (AR). In contrast to the large number of genes and loci (at least 20) identified in human autosomal dominant cataract, only two genes, *CRYAA* and *LIM2*, and two loci with as yet unidentified genes have been implicated in AR cataract. Eight families were recruited, five of which are consanguineous, with early onset bilateral cataract and at least 2 affected members, in order to further localize and identify the genes responsible for AR cataract. STRP markers for the previously published AR loci (3p21.1 - p21.3, 9q13-q22, 19q13.4, 21q22.3) were chosen for genotyping. For those families not excluded from 19q and 21q, the coding sequences of *LIM2* and *CRYAA* will be screened by SSCP analysis. The disease in one family cosegregated with markers of the 3p loci, but did not narrow the published disease interval. The remaining families have been excluded from three AR loci by genotype/haplotype analysis and SSCP(*CRYAA*). This study provides further evidence for genetic heterogeneity in the AR form of cataract. The molecular characterization of AR cataract is essential in order to better understand the complex cascade of events modulating lens opacification in the more common age-related cataract.

Genetics of X-linked congenital stationary night blindness (CSNB): summary of mutation analysis. *N.T. Bech-Hansen¹, R. Tobias¹, T. Rosenberg², J. Robitaille³, K.D. Unger¹, A. Zaprzelski¹, P.A. Farndon⁴, M. Schwartz², D.G. Birch⁷, J.R Heckenlively⁵, R.G Weleber⁶.* 1) Med Genetics, Univ Calgary, Calgary, AB, Canada; 2) National Eye Clinic, Denmark; 3) Dept. Ophthalmology, Dalhousie Univ, Halifax, NS, Canada; 4) Dept of Genetics, Univ of Birmingham, UK; 5) Jules Stein Eye Institute, Los Angeles, CA; 6) Casey Eye Clinic, OHSU, OR; 7) Retina Foundation of the Southwest, Dallas, TX.

X-linked CSNB represents a rare disorder of retinal neurotransmission. Patients present with a variable phenotype of reduced visual acuity, which may include myopia, night blindness, nystagmus, or strabismus; they all have negative electroretinograms (ERGs). Genetic analysis has revealed genetic heterogeneity, CSNB1 and CSNB2, which correlates with the clinical entities of complete CSNB and incomplete CSNB. Moreover, the causative genes have been identified as *NYX* (CSNB1) and *CACNA1F* (CSNB2). Using direct DNA sequencing, we have expanded the analysis of these two genes to a total of 80 families with X-linked CSNB and identified a total of 53 unique mutations. In patients with complete CSNB from 25 families, we have detected 9 missense, 5 in-frame insertion/deletion, 1 splice-site, 1 nonsense, and 1 frame-shift mutations in *NYX*, which encodes the GPI-anchored proteoglycan nyctalopin. In patients with incomplete CSNB from 44 families, we detected a total of 27 different mutations in *CACNA1F* (7 nonsense, 5 frameshift, 4 splice-site, 7 missense, and 1 in-frame insertion/deletion mutations) which are each predicted to alter or lead to a loss of function of the alpha-1-F subunit that is part of a retinal-specific voltage-gated L-type calcium channel. In addition, patients from 10 different Danish families diagnosed with Aland Eye disease (AED) and a British family diagnosed with Aland Island Eye disease (AIED) were also found to have mutations in *CACNA1F* (5 missense, 1 in-frame deletion, 1 splice-site, 3 nonsense, and 2 frameshift mutations). These findings provide further support for the causative role of *CACNA1F* and *NYX* in X-linked CSNB conditions, including AED and AIED. Supported in part by CIHR, and Foundation Fighting Blindness (Canada).

Fine mapping of the GLC1C locus in a large Greek pedigree segregating primary open angle glaucoma. G. Kitsos¹, K. Rust², R. Sykes², J.R. Samples², E. Economou-Petersen³, M. Grigoriadou⁴, G. Aperis⁴, K. Psilas¹, M.B. Petersen⁴, M.K. Wirtz². 1) Univ. of Ioannina, Greece; 2) Casey Eye Inst., Oregon Health Sciences Univ., Portland, Oregon; 3) Drakopoulion Blood Bank Center, Athens, Greece; 4) Inst. of Child Health, Athens, Greece.

Glaucoma is a heterogeneous group of disorders, the majority of which are associated with an open, normal appearing anterior chamber angle and are termed open angle glaucoma (OAG). OAG is likely to be a genetically heterogeneous disorder that results from the interaction of multiple affected genes and environmental influences. Six loci have so far been mapped by linkage analysis in families with autosomal dominant mode of inheritance of OAG, GLC1A-GLC1F. Only one gene has so far been identified, the TIGR gene responsible for the GLC1A locus. Mutations in the TIGR gene have been detected in most familial cases of juvenile onset OAG as well as in a small proportion of adult onset OAG patients, also known as primary OAG (POAG). We have previously mapped the GLC1C locus to chromosome 3q21-q24 in one large North American pedigree and one large Greek pedigree with autosomal dominant POAG. Haplotype analysis suggested independent origin of the genetic defect in the two families. We here present refined mapping of the GLC1C locus in the Greek family by including more family members and analyzing more markers in the chromosome 3q inclusion region. A total of 89 individuals from 4 generations were clinically examined and genotyped. Clinical findings in affected individuals included characteristic glaucomatous changes in the visual field, increased cup to disc ratio, and intraocular pressure before treatment more than 21 mmHg, with age at diagnosis 31 years and older. DNA microsatellite polymorphism and haplotype analysis together with identification of recombinant individuals narrowed the GLC1C critical region to the interval between markers D3S3637-D3S1555. Two individuals were homozygous for the partial disease haplotype D3S3637-D3S3694 and were unaffected at the age of 65 and 63 years, respectively. This study represents a step further towards the identification of the GLC1C disease causing gene.

RS1 mutations causing X-linked Juvenile Retinoschisis lead to intracellular retention of mutant retinoschisin. D.

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X-linked retinoschisis results in visual loss in early life with the characteristic abnormality of schisis within the inner retina. Patients often present at school age but can present within the first few months of life. Many missense and protein truncating mutations of the causative gene RS1 (encoding retinoschisin) have been identified but the disease is variable even within families and severity is not mutation-dependent. Retinoschisin is secreted and predicted to have a globular conformation. Missense mutations would be expected to interfere with protein folding leading to an abnormal conformation and elimination by intracellular protein degradation. To test this we have expressed 7 pathological RS1 mutations (L12H, C59S, G70S, R102W, G109R, R141G, R213W) in Cos-7 cells and investigated their intracellular processing. Constructs of WT RS1 and RS1 mutations in pcDNA3.1 and fusions with EGFP were generated and expressed in Cos-7 cells. Using immunoblotting and confocal fluorescence microscopy we have shown normal secretion of WT RS1, but either reduced (C59S, R141G) or absent (L12H, G70S, R102W, G109R and R213W) secretion of mutant RS1 and retention in the endoplasmic reticulum (ER). We found that WT and mutant RS1 are associated with the molecular chaperones Bip (GRP78 from the HSP70 family) and PDI. In addition, we have shown that L12H RS1 is degraded by the proteasome pathway and in vitro transcription/translation revealed the lack of cleavage of its signal peptide. Our results indicate the pathological basis of RS1 is intracellular retention of retinoschisin and this may explain why disease severity is not mutation dependent.

Mutation Screening of *RPGR* in Male Patients with X-Linked or Sporadic Forms of Retinitis Pigmentosa or Cone-Rod Dystrophy. *A.L. Radak*¹, *F.Y. Demirci*^{1,2}, *B.W. Rigatti*², *E.I. Traboulsi*³, *T. Alitalo*⁴, *T.S. Mah*^{1,2}, *M.B. Gorin*^{1,2}. 1) Dept of Human Genetics, GSPH, Univ of Pittsburgh, Pittsburgh, PA; 2) Dept of Ophthalmology, School of Medicine, Univ of Pittsburgh, Pittsburgh, PA; 3) Center for Genetic Eye Diseases, Cole Eye Inst, Cleveland Clinic Foundation, Cleveland, OH; 4) Dept of OB/GYN, Helsinki University Hospital, Helsinki, FINLAND.

Retinitis Pigmentosa (RP) refers to a heterogeneous group of phenotypically similar hereditary retinal disorders described in several inheritance patterns, as well as sporadically. X-linked (XL) RP (XLRP) is a generally severe form of RP. The *RPGR* (retinitis pigmentosa GTPase regulator) gene isolated from *RP3* region (Xp21.1) was found to be mutated in up to 70% of XLRP families. This includes mutations in exon ORF15 (an alternative 3' terminal exon of *RPGR*) that contains a mutational hot spot. In addition, exon ORF15 mutations have been shown to cause XL-atrophic macular degeneration and a subset (COD1) of XL-cone-rod dystrophy (XLCRD). Using direct PCR sequencing, we screened *RPGR* exons 1-14 and ORF15 for mutations in samples from 4 XLRP and 12 XLCRD families and a total of 20 North American sporadic male patients with either RP or CRD. We found mutations in two XLRP samples (ORF15+483_484delGA and IVS1+1G>A) and in one sporadic RP sample (1404C>T), although analyses of additional samples are still underway. Despite a sampling bias created by our previous efforts to recruit COD1 families (corresponding *RPGR* mutations published elsewhere), more than half of our current XLCRD families have not been mapped to *COD1* and/or lack *RPGR* mutations, supporting the importance of genetic heterogeneity. Further mutation analysis in sporadic samples will help to determine the rate at which mutations in *RPGR* affect these patients. Our data supports the involvement of *RPGR* in sporadic cases, although different criteria in the selection of cases for *RPGR* screening may result in reporting different frequencies. The comparison of phenotypes of *RPGR* mutation positive patients versus mutation negative patients may provide indicators for the prediction of sporadic cases at high-risk for *RPGR* mutations and serve as a valuable guide for future genetic counseling.

Inherited retinopathy causing mutations in the AIPL1 protein interfere with its interaction with the cell-cycle control protein, NUB1. *M.M. Sohocki¹, C.G. Castellano¹, A.F. Sorensen¹, D.T. Akey².* 1) Dept Ophthal, Columbia Univ, New York, NY; 2) Dept of Envrmtl Hlth, Univ Cincinnati, OH.

Mutations in the aryl hydrocarbon receptor-interacting protein-like 1 gene have been found in patients with Leber congenital amaurosis (LCA), a severe, early onset form of inherited blindness. The AIPL1 protein has considerable homology with the aryl hydrocarbon receptor-interacting protein (AIP), which helps to mediate the stabilization and nuclear transport of the aryl hydrocarbon receptor as part of a complex with hsp90. To determine the normal function of AIPL1 and to better understand how mutations in this gene cause disease, we performed a yeast two-hybrid screen to identify AIPL1-interacting proteins. One of the identified interacting proteins corresponds to Nub1 (NEDD8 Ultimate Buster-1), which is thought to control many biological events, including cell-cycle progression. In addition, constructs were designed to determine what regions of the AIPL1 protein were involved in the interaction. To determine the potential impact of LCA-causing mutations on the interaction, nine LCA-associated mutations were introduced into the AIPL1 construct. The ability of the mutant AIPL1 proteins to interact with the NUB1 protein was determined through quantitative b-galactosidase assays and growth in liquid dropout media. While each deletion construct was capable of interacting with NUB1, the construct encoding the C-terminal region of AIPL1 appeared to have the highest binding activity. This is consistent with the finding that the sequence required for interaction of AIP with hsp90 is within the final 20 amino acids of AIP. The majority of the mutations significantly reduced the interaction between these proteins. Therefore, we hypothesize that the majority of AIPL1 mutations cause inherited retinopathy due to loss of function. Due to the likelihood that AIPL1 is functioning as part of a multi-protein complex, it is possible that the mutations not associated with reduced interaction with NUB1 affect the interaction with another component of the AIPL1 complex. Alternatively, it is possible that these mutations result in mislocalization of the protein within the cell.

Abnormal X chromosome inactivation in female patient with partial hypoxanthine-guanine

phosphoribosyltransferase (HGPRT) deficiency. *L. Dvorakova¹, J. Hujova¹, R. Dobrovolny¹, L. Stolnaja¹, E.*

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Partial deficiency of hypoxanthine-guanine phosphoribosyltransferase (HGPRT) described as Kelley-Seegmiller syndrome is an X-linked recessive disorder of purine metabolism associated with gout and urolithiasis. This phenotype occurs almost exclusively in males. We follow a girl who presents gouty arthritis of big toe and hyperuricaemia from the age of 9 years. Biochemical studies revealed partial HGPRT deficiency. Direct sequencing of PCR products prepared from genomic DNA revealed that our female patient inherited mutation c.158T>C (V53A) from her father. This mutation located in the 3rd exon of HGPRT gene was previously described in a patient with similar phenotype. The proband's sister and grandmother (father's mother) are also heterozygous for V53A, although they are asymptomatic. The severe phenotype of the female proband can be explained by the following: 1. There is another mutation on the second allele, or 2. there is an unfavorable skewing in X-inactivation towards the mutant allele. To examine the first possibility we cloned both alleles into a plasmid. The entire sequence of the allele, which did not carry the mutation c.158T>C, was sequenced and no other mutation was detected. X-inactivation analysis in DNA from peripheral leukocytes suggests that our female patient has preferentially activated the mutant allele inherited from her father. On the contrary, her asymptomatic sister and grandmother use almost exclusively the normal allele. The presented data suggest, that in girls with unexplained hyperuricaemia and gout the partial HGPRT deficiency has to be taken into account. While biochemical tests performed in heterozygotes may fail because of X-inactivation status, mutation analysis is a reliable tool for carriers identification. (Supported by grants NE 6557-3-01 of the Czech Ministry of Health and MSM-111100005 of the Czech Ministry of Education).

Molecular basis of Oro-facio-digital type I syndrome. *B. Franco, G. Giorgio, A. Barra, A. Ballabio, M. Ferrante.*
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Oro-Facio-Digital type 1 (OFD1) is an X-linked dominant condition characterized by polycystic kidneys and malformations of the face, oral cavity, digits, and brain. We identified the gene responsible for this disorder, OFD1, and showed that it is an anonymous protein characterized by five predicted coiled-coil domains. We have now collected 23 OFD1 patients (8 familial and 15 sporadic) and found mutations in all cases. These include 3 missense, 14 frameshifts, 3 splice site and 3 non sense mutations. The mutations are distributed along the protein although 6 out of 23 (26%) are located in exon 3. To gain insight into the pathogenetic mechanism underlying OFDI, sub-cellular localization experiments were performed in transfected COS7 cells on wild type and mutated forms of the OFDI protein. Our study showed that the wild type OFD1 displays a punctate distribution, concentrating in speckles of variable size and uniformly distributed in the cytoplasm. On the other hand, constructs carrying the mutated forms showed a more diffuse and granular pattern when compared to the wild-type, as the cytoplasmic speckles disappear, resulting in a more homogeneous distribution of the protein in the cytoplasm. Co-localization experiments with markers defining different organelles have been so far unsuccessful. In order to test whether OFD1 could act via a protein-protein interaction mechanism through its coiled-coil domains, interaction mating and co-immunoprecipitation experiments were performed. Our results showed that the protein does homo-interact through its central region where most of the coiled-coils are located. Two hybrids experiments are currently in progress to identify potential OFD1 interactors. A null targeted mutation of the OFD1 gene is being generated in mouse: preliminary analysis of the chimeras revealed the presence of features typical of the human condition. The availability of a mouse model will be critical for the definition of the OFD1 biological role and to gain insights in the pathogenesis of this developmental disorder. Special thanks to clinicians, who provided patients, and who were not included in the author's list due to space limitations.

Mutations in the X-linked dominant type (CDPX2) of chondrodysplasia punctata. *S.E. Chavez¹, R.I. Kelley², L.E. Kratz², A.B. Metzenberg¹.* 1) California State University, Northridge, CA; 2) Kennedy Krieger Institute Baltimore, MD.

Mutations in the X-linked dominant type (CDPX2) of chondrodysplasia punctata. X-linked dominant chondrodysplasia punctata (CDPX2, OMIM #302960) is a rare disorder of cholesterol metabolism, one of a group of such disorders affecting skin and bone development. CDPX2 is characterized by punctate calcification in cartilage, asymmetric rhizomelia, cataracts, patchy alopecia, ichthyosis and atrophoderma. The phenotype includes high levels of intermediate metabolites of cholesterol biosynthesis in plasma and cultured fibroblasts, which results from a deficiency of sterol-D8, D7-isomerase, that is encoded by the EBP gene, located on Xp11.22-23. Here we present the results of biochemical and sequence analyses of 8 unrelated females with clinical diagnoses of CDPX2, and elevated levels of cholest-8(9)-en-3-ol and 8-dehydrocholesterol. Mutations were found in EBP in 7 of the 8 samples. Of these, two were previously reported missense mutations, predicted to cause the substitutions R147H (seen in two of our patients), and E80K. One previously reported nonsense mutation, W61X, was observed in one patient. Three mutations are novel, and include a missense mutation, P200F, a nonsense mutation, W47X, and one splice donor mutation in intron 2, nt301+1 G>A. We found no evidence for genotype-phenotype correlation in these patients. The patient for which no mutation was found in the coding regions or splice junctions of the EBP gene may have a mutation in a regulatory region or in an unknown gene affecting EBP activity. Through this report and others, a pattern is emerging showing both the importance of biochemical analysis in patients with a clinical diagnosis, and the relatively high frequency of novel mutations in the EBP gene requiring molecular diagnosis through sequence analysis.

Hemiduplication of body organs: A possible disorganization-like syndrome. *M. Thomas¹, K. Chong¹, B. Mullen², A. Pai¹.* 1) Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hosp, Toronto, ON, Canada; 2) Dept of Pathology, University of Toronto, ON, Canada.

The mouse mutant disorganization was first fully described by Hummel (158, 1959). Who stated that it received its name from the fact that it disrupts the orderly process of organogenesis and induces a great variety of developmental anomalies in structure derived from germ layers. We report a fetus with duplication of the external and internal body organ of the right side of the body which resemble the disorganization syndrome reported in the mouse mutant. This was the first pregnancy to a non-consanguineous couple of Anglo-Saxon origin, with negative family and pregnancy history. Fetal ultrasound at 20 weeks gestation revealed two vessel cord, bilateral club foot and right diaphragmatic hernia. The pregnancy was terminated and the autopsy showed hypoplasia of the right face, sternum, right ribs, trunk, labia majora and sternum. There were duplication of right arm, right clavical, right lung, right stomach, partial duplication of the small bowel, duplication of the right iliac wing, right adrenal gland and right kidney. There was a cloacal tag, right diaphragmatic hernia with deviation of the heart to the left, and pulmonary hypoplasia. The female fetus had a bicornate uterus, with segmentation defect in the upper thoracic spine and 11 pairs of ribs. The fetus also had bilateral talipes equinovarus, sandal gap on left foot, anular pancreas and melodysplasia of the spinal cord. There was pterygium involving the right elbow and right knee and a single umbilical artery. No abnormalities were detected in the CNS. The mouse disorganization mutant presents with complete limb duplication as well as other abnormalities. Several human cases with similar findings have been reported mainly with limb duplication and hamartomatous lesions in different parts of the body. To the best of our knowledge, such an extensive hemiduplication of different body organs has not been reported. We think that the findings are consistent with the disorganization-like syndrome.

Casamassima-Morton-Nance syndrome : Another case and review of the literature. *C. Thauvin-Robinet Jr¹, L. Laurent², S. Lionnais³, T. Rousseau³, N. Nadal⁴, F. Mugneret⁴, C. Durand⁵, P.D. Turnpenny⁶, P. Sagot³, L. Faivre¹.* 1) Centre de Genetique, Hopital d'Enfants, Dijon, France; 2) Laboratoire d'anatomopathologie, Hopital du Bocage, Dijon, France; 3) Clinique gynecologique et obstetrique, Maternite du Bocage, Dijon, France; 4) Laboratoire de Cytogenetique, Hopital du Bocage, Dijon, France; 5) Service de Radiologie, Hopital d'Enfants, Dijon, France; 6) Molecular Genetics, School of Postgraduate Medicine and Health Sciences, Barrack Road, Exeter, United Kingdom.

Casamassima-Morton-Nance syndrome (CMN; MIM 271520) is a rare disorder characterized by the association of spondylocostal dysostosis with "crab-like" appearance of the thoracic skeleton, various urogenital abnormalities and anal atresia. Only five cases with CMN have been reported in the literature. Differential diagnose are represented by spondylocostal dysostosis syndromes with "crab-like" appearance of the thoracic skeleton, including Jarcho-Levin syndrome, autosomal dominant spondylocostal dysostosis, and spondylothoracic dysostosis. Here, we report on a fetal case of CMN born from Caucasian non consanguineous parents. Ultrasound survey at 12 weeks of gestation showed severe growth retardation and suspicion of scoliosis. Autopsy performed after spontaneous death at 14 weeks of gestation revealed major scoliosis, asymmetric trunk, anal atresia and agenesis of external and internal genitalia, bladder and kidneys. Radiological features included hemivertebrae and rib abnormalities with crab-like appearance of the thoracic skeleton. These findings fit with the diagnosis of Casamassima-Morton-Nance syndrome. Reviewing the literature, apart from the characteristic clinical features, the five reported cases with CMN also presented atypical signs such as midthoracic meningocele (1/5), polymicrogyria (1/5), preaxial polydactyly (1/5), hypoplastic right femur (1/5) and sacral agenesis (1/5). Sequence analysis of the DLL3 gene involved in Jarcho-Levin syndrome is in progress in the present reported case.

Osteocraniostenosis : Lethal dwarfism with thin bones, craniosynostosis and splenic hypoplasia. Report of a new

case. *S. Patrier*¹, *A. Rossi*², *A. Diguert*³, *D. Eurin*⁴, *E. Verspyck*³, *A. Laquerriere*¹, *A. Verloes*⁵. 1) Dept of Pathology, University Hospital, Rouen, France; 2) Dept of Genet, EFS-Normandie, Bois-Guillaume, France; 3) Dept of Obstetrics and Gynecology, University Hospital, Rouen, France; 4) Dept of Radiology, University Hospital, Rouen, France; 5) Dept of Genet, University Hospital Robert Debre, Paris, France.

A 25 year-old Caucasian woman was referred to our hospital for major intrauterine growth retardation at 20 weeks gestation. Ultrasound examination showed micromelic dwarfism and facial dysmorphism. The parents refused to terminate pregnancy . The fetus died at 36 weeks. The female fetus weighed 447g (compare to an expected average of 2220g). Crown-heel distance was 25cm, crown-rump 19cm, OFC 24cm (compare to an expected average of 45, 31 and 32cm respectively). There was a major micromelic dwarfism with acromicria and stubby as well as short fingers, and hypoplastic nails. The skull was scaphocephalic with a mild cloverleaf deformity and wide prominent forehead. Flat face with microphthalmia and hypertelorism, short nose, short philtrum and micrognathia were noted. Radiological examination showed very thin and sclerotic long bones, and diffuse hypomineralisation. Diaphyses were dense and thin like fishbones with flared metaphyses. Hands showed brachyphalangy and brachymetacarpus. The fibulae were filiform. The cranial vault was not ossified and the spine slightly mineralized with platyspondyly. At the autopsy, the vault was totally membranous. Spleen was hypoplastic. Histological examination displayed severe abnormalities of enchondral ossification. Epiphyseal cartilage contained few chondroblasts. Metaphyseal plate showed rare columns of hypertrophied cartilage, short and irregular primary bone trabeculae. The diaphysis was entirely filled with dense osteoid tissue without bone marrow. This observation matches the description of cases reported by Verloes and al. describing a rare and multiple congenital abnormalities syndrome called osteocraniostenosis. Homogeneity and genetic origin of this rare form of osteochondrodysplasia remains unclear. This fetus observation is worth being reported as an extremely severe form of this syndrome.

Cell free fetal DNA is detectable in the CSF of some pregnant women. *R.M. Angert¹, E.S LeShane¹, R.W. Yarnell², D.W. Bianchi¹*. 1) Genetics, Tufts-NE Medical Center, Boston, MA; 2) Anesthesia, Tufts-NE Medical Center, Boston, MA.

Introduction: Cell-free fetal DNA is detectable in maternal plasma. Its origins, distribution, and metabolism remain a mystery but are an area of active inquiry. We hypothesized that fetal DNA could cross the maternal blood-brain barrier and would be detectable in cerebrospinal fluid (CSF).

Materials and Methods: With IRB approval we obtained up to one mL of discarded CSF from 2 women who were having spinal anesthesia for cerclage placement (first trimester), 20 women who were undergoing routine cesarean section (third trimester, antepartum) and 4 women undergoing tubal ligation (third trimester, 24 hours post-partum). DNA was extracted from spun CSF and amplified using real time PCR for *DYS-1* (representing fetal) and *b-globin* (representing total) to quantitate the cell free DNA.

Results: 26 samples of maternal CSF were collected. There were 19 males, 5 females, one set of triplets, and one unknown. *b-globin* DNA sequences were detected in each of the samples [91 ± 115 genome equivalents(GE)/mL]. In one routine cesarean section with a male fetus there was a significant amount of male cell free DNA present in the CSF (7 GE/mL). A sample from a patient bearing triplets (male, male, female) undergoing cesarean section had male cell free DNA detected (9 GE/mL). One post-partum tubal ligation patient whose baby was male had a significant amount of male cell free DNA present in the CSF (6 GE/mL). The remainder of the CSF samples had no detectable male cell free DNA.

Conclusion: Total cell free DNA is detectable in maternal CSF samples. Male cell free DNA, presumably of fetal origin, was detectable in 3 out of 20 samples (15%). This suggests that male fetal cells or cell free DNA may cross the blood brain barrier in pregnant or post-partum women.

Prenatal diagnosis for deletion of SMN gene: occasional observation? *V. Sokolnik, S. Shreder, A. Spector, G. Tsukerman.* Department for Prevention of Hereditary Diseases, Institute for Hereditary Diseases, Minsk, Belarus.

The survival motor neuron (SMN) gene presents in variable number of two almost identical copies (SMN1-telomeric copy, SMN2-centromeric copy). SMN1 gene is absent in the majority of patients with SMA, whereas SMN2 gene copy number modifies the phenotype. Both genes undergo alternative splicing, however, the full-length (FL) transcript is the major product of SMN1, while SMN2 expresses reduced level of full-length transcript and abundant level of transcripts lacking exon 7 (Lefebvre et al., 1995;). The tissues from SMA-fetus with deletion of SMN1 have more transcripts containing exon 7 than peripheral blood of SMA patients (Jong et al., 2000). SMNDelta7 is potently proapoptotic, causing increased cell-specific neuronal apoptosis, whereas full-length SMN protects primary neurons and differentiated neuron-like stem cells from virus-induced apoptotic death (Kerr et al., 2000). SMN2 deletions may be not biologically inert because can alter the SMNDelta7/FL ratio. Our previous studies, with using of the SSCP, have demonstrated that SMN1 was deletion changed in about 82% of Belarus SMA patients, 1,6% parents, while the SMN2 was deleted in 2,6% SMA patients and in 4,8% parents. About 9,5% random control newborns have deletions in SMN2 that be detected by SSCP analysis of amplified DNA from dried blood spots. Now we report the result of restriction site assay study of chorionic villi SMN genes. The patient was the 11-week fetus with karyotype 46 XX. The mother, 24-year old, is healthy. The father, 23-year old, has spina bifida and congenital heart defect. Ultrasound examination was performed at 11th week of gestation and the patient showed an exencephaly. Under consideration of the poor prognosis the termination of pregnancy was made in accordance with the parents. Analysis of SMN genes from chorionic villi revealed that the fetus was deleted for SMN2 exon 7. As far as we know, it is a first observation of SMN2 gene deletion in exencephaly a condition, in which defect in neural tube closure resulting in an overgrowth of undifferentiated neural tissue? This condition is usually found in embryos as an early stage of anencephaly.

Microchimerism and cervical cancer. *K.L. Johnson, D.H. Cha, H. Stroh, D.W. Bianchi.* Department of Pediatrics, Division of Genetics, Tufts-New England Medical Center, Boston, MA.

Background: Fetal cell microchimerism has been shown to be associated with autoimmune disease through a graft-versus-host like response. Microchimerism has also been described in non-autoimmune diseases, such as infectious hepatitis. Microchimerism may result from other sources, including through the persistence of spermatozoa or semen leukocytes following sexual intercourse. The current study was performed to determine if microchimerism is seen in cervical cancer.

Methods: Fluorescence in situ hybridization (FISH) using X and Y chromosome-specific probes was performed on paraffin-embedded cervical tissue sections to allow for the identification of microchimeric male cells within a tissue specimen from a female. Sections from five women with cervical cancer and known pregnancy histories that included at least one live born male were analyzed.

Results: Male cells were observed at low numbers (2-5 cells per section) in one patient with squamous cell carcinoma and in one patient with adenocarcinoma. In both cases, the tissue sections that were analyzed were ~ 1 x 2 cm in area. Microchimeric cells were not observed in the other cases, all of which were represented by smaller specimens (e.g. ~ 1 x 5 mm area).

Conclusions: Microchimerism may be associated with cervical cancer, possibly resulting from fetomaternal cell trafficking or from the presence of semen-associated nuclei. These data, combined with additional data from our laboratory, suggest that microchimerism may be a widespread phenomenon involving many types of disease, including cancer.

22q11 Deletions in Fetuses with Conotruncal Anomalies or Interruption of the Aortic Arch: morphogenetic mechanisms and implications for patient management. *M. Gentile^{1,2}, P. Volpe², M. Marasini³, A.L. Buonadonna¹, M.C. Valenzano¹, F.M. Boscia².* 1) Medical Genetics, IRCCS S de Bellis, Castellana , Bari, Italy; 2) Obstetrics and Gynecology, A.O. Di Venere, Bari, Italy; 3) Pediatric Cardiology, IRCCS Gaslini, Genoa, Italy.

We investigated 100 cases of conotruncal anomalies (CTA) or interrupted aortic arch (IAA), identified in prenatal age, in order to detect the prevalence of 22q11 microdeletion, evaluate the relationship between embryological development of these anomalies and microdeletion of 22q11, and make a comparison between US/FISH results and neonatal clinical findings and outcomes. Prenatal echocardiography and FISH for the DiGeorge critical region (22q11) were performed in all cases. In positive cases the findings were confirmed by autopsy (6 cases) or at post-natal echocardiography and surgery (12 cases). On fetal echocardiography we detected 9 cases of IAA, 12 of Truncus Arteriosus (TA), 31 of Tetralogy of Fallot (TOF), 12 of Pulmonary Atresia with Ventricular Septal Defects (PA with VSD), 15 of Double Outlet Right Ventricle (DORV) and 21 of Transposition of Great Arteries (TGA). FISH detected microdeletions in 22q11 in 18 of 100 fetuses. Our study confirms the feasibility of prenatal diagnosis of heart defects, based on echocardiographic examination, and the high prevalence of 22q11 microdeletion in cases of CTA or IAA. Furthermore our data support the role of 22q11 microdeletion in clinical management of fetuses affected by conotruncal and/or aortic arch anomalies.

Truncus arteriosus in a 45,X male fetus with SRY;autosome translocation. A.K. Yenamandra¹, L. Hentze¹, E. Krim², I. Sadr³, A. Ahmed⁴, W. Fried², P. Koduru¹, L. Mehta³. 1) Cell Genetics, North Shore Univ. Hosp., Manhasset, NY; 2) Dept. Ob/Gyn., North Shore Univ. Hosp., Manhasset, NY; 3) Med. Genetics & Ped. Cardiology, Schneider Children's Hosp. at North Shore, Manhasset, NY; 4) Dept. Pathology, Tampa General Hospital, Tampa, Fla.

A 26 y.o G1P0 woman at 30 weeks gestation was found to have fetal growth retardation and oligohydramnios on ultrasound. The fetus had a single umbilical artery and a heart defect was suspected. Fetal echocardiogram showed truncus arteriosus type I (TA) and a large ventricular septal defect (VSD). Amniotic fluid interphase FISH showed a single X chromosome signal with normal signals for chromosomes 13, 18 and 21. Turner syndrome was presumed, however the fetus had male genitalia on sonogram. Karyotype was confirmed to be 45,X and a FISH study with the SRY probe (located at Yp11.3) showed translocation of SRY to the terminal region of the long arm of chromosome 4. A diGeorge syndrome critical region (22q11.2) FISH study was normal. The biophysical profile of the fetus worsened and the patient opted to undergo late termination of pregnancy. Autopsy confirmed TA type I, a dysplastic semilunar valve, membranous VSD and a left persistent superior vena cava. The right kidney and ureter were hypoplastic. No other birth defects or dysmorphisms were noted. Normal male genitalia were present. Both parents had normal blood chromosome studies. Previous reports of 45,X males with Y;autosome translocations include patients with mental retardation (Y;15), cri-du-chat syndrome (Y;5), trisomy 18 (iso18p) and features of 18p deletion (Y;18). In this case the Y;4 translocation had to be followed by loss of the rest of the Y chromosome. An abnormality at the site of translocation on chromosome 4q could have caused the heart defects as no other etiology was apparent. Though left-sided heart defects are well known in 45,X Turner syndrome patients, TA is only reported in one other patient. Chromosome 4q deletions are associated with heart defects, though not specifically TA. Submicroscopic loss or intersection or inactivation of genes at the site of SRY translocation may determine phenotype in 45,X males with SRY;autosome translocations.

Matthew-Wood syndrome: Prenatal diagnosis and further delineation of a rare condition. *H. Sroka*¹, *G. Ryan*², *S. Keating*³, *A. Pai*¹, *D. Chitayat*¹. 1) Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hosp, Toronto, ON, Canada; 2) Dept. of Obstetrics and Gynecology, Mount Sinai Hosp, Toronto, ON, Canada; 3) Dept. of Pathology, University of Toronto, Toronto, ON, Canada.

Matthew-Wood syndrome is rare condition consisting of pulmonary agenesis, microphthalmia/anophthalmia, and a diaphragmatic abnormality. This unique syndrome is presumably autosomal recessive based on a case report of affected siblings born to unaffected parents (Seller et al. 1996). We report the prenatal diagnosis of Matthew-Wood syndrome based on the fetal ultrasound findings of primary pulmonary hypoplasia, microphthalmia, eventration of the diaphragm and a cardiac abnormality at 25 weeks gestation. The mother was a 26-year-old G4P3L2 woman of English/French Canadian descent and her husband was 27 years old and of French Canadian/English descent. The couple was healthy and non-consanguineous. The couple has two healthy daughters and lost a pregnancy at 34 weeks gestation due to an entangled cord. A fetal ultrasound done at 24.5 weeks gestation in the couples fourth pregnancy revealed left diaphragmatic eventration, bilateral microphthalmia/anophthalmia and cardiac abnormality. Amniocentesis demonstrated a normal female karyotype (46, XX) with no deletion detected at 22q11.2. A fetal echocardiography revealed that the heart occupied most of the left chest. There was a subaortic VSD with mild degree of aortic overriding which could present a mild TOF or a VSD with aortic overriding. Fetal MRI showed bilateral anophthalmia and a cystic structure in the region of the left orbit. The left lung was not identified, the right lung was very small and there was a left diaphragmatic eventration. A female was delivered at 38 weeks gestation and died shortly after birth. An autopsy showed bilateral anophthalmia, diaphragmatic eventration, a hypoplastic heart, hypoplastic lungs more marked on the left than the right, a bicornuate uterus, and a gliopendymal cyst of the corpus callosum.

Ambiguous genitalia in a prenatally diagnosed case of 45,X/46,X,psu idic(Y)(p11.32)karyotype. *S.K. Dhandha, E.W. McPherson, W.A. Hogge.* Dept Genetics, Magee Hosp, Univ Pittsburgh, Pittsburgh, PA.

We report a prenatally diagnosed case of a mosaic karyotype with a 45,X cell line and another cell line consisting of 46,X,idic(Y) karyotype. Prenatally, the majority of the cells (90%) were 45,X on amniocentesis. Therefore, the family was counseled on the Turner phenotype, with the possibility of ambiguous or male genitalia. Follow up ultrasounds showed ambiguous genitalia.

At birth the infant had a bifid scrotum with palpable testes, hypoplastic penis, and hypospadias. Peripheral blood karyotype showed a reversal of the proportions of the cell lines, with 80% of cells containing the extra idic(Y). This case illustrates the difficulty of counseling patients prenatally with mosaic karyotypes (especially those involving sex chromosomes) due to uncertainties involving tissue specific mosaicism. It also raises issues of gender assignment in infants with ambiguous genitalia.

The clinical utility of karyotyping spontaneous abortions. *A.L. Byrnes¹, W.A. Hogge^{1,2}, M.C. Lanasa¹, U. Surti³.* 1) Department of Genetics, Magee-Womens Hospital, Pittsburgh, PA; 2) Department of Obstetrics, Gynecology, and Reproductive Sciences, Magee-Womens Hospital, Pittsburgh, PA; 3) Department of Pathology, Magee-Womens Hospital, Pittsburgh, PA.

Objective: Recurrent pregnancy loss (RPL) affects approximately 1% of couples. Karyotyping of spontaneous abortion material has been advocated as a cost-effective tool in the evaluation of RPL. The objective of this study was to assess the clinical utility of routinely karyotyping spontaneous abortion material. Study Design: We retrospectively reviewed the records of the Pittsburgh Cytogenetics Laboratory from January 1, 1998 through December 31, 2001 for tissues from spontaneous losses at 20 weeks gestation or less for which complete cytogenetics and pregnancy medical records were available. Pregnancy history and maternal demographics were obtained from hospital records. Results: There were submitted samples from 517 individuals of which 28 (5%) failed to grow in culture. Overall, 56% of samples were abnormal. Fifty-two percent of normal results were male. In samples from pregnancies at 13 weeks or less the rate of abnormality was 69%. When analyzed by maternal age, the rate of abnormality in first trimester losses was 57% in women less than 35 years of age, and 82% in those 35 years of age or older. For all losses, there was no difference in the rate of abnormality when comparing first loss versus two or more losses, first pregnancy versus two or more pregnancies, or the presence or absence of at least one live birth. In 82 women with presumed non-chromosomal etiology for a first trimester loss, the rate of chromosome abnormality was 65%. Conclusion: Chromosome abnormalities are the etiology for pregnancy loss in 50-80% of cases, depending on maternal age. There is no association with number of previous losses. Karyotyping of first trimester spontaneous losses beginning with the patient's second loss would provide precise etiologic information, and decrease the number of evaluations necessary for presumptive recurrent pregnancy loss.

TRENDS IN LIVE BIRTH PREVALENCE OF CONGENITAL ANOMALIES 1979-1999 : THE IMPACT OF PRENATAL DIAGNOSIS. *Y. ALEMBIK, M.P. ROTH, B. DOTT, C. STOLL.* Medical Genetics, Hpital de Hautepierre, STRASBOURG, France.

Objectives To describe the impact of prenatal diagnosis on the birth prevalence of congenital anomalies during 21 years (1979-1999) in a well defined population. **Design** A descriptive population-based study. **Setting** Northeastern France (13,500 births per year). **Methods** Analysis of data from multiple sources on births and terminations of pregnancy after prenatal diagnosis of congenital anomalies in 265,679 consecutive pregnancies of known outcome. The study period was divided into 3 subgroups 1979-88, 1989-93 and 1994-99. **Results** Between 1979 and 1988 and 1993 and 1999 prenatal detection of congenital anomalies increased from 11.7% to 25.5% and to 31.9%. Termination of pregnancy (TOP) increased in the same proportions during the 3 time periods. However the increase of TOP was much higher for chromosomal anomalies than for non chromosomal congenital anomalies : 21.7, 43.9 and 64.0 vs 4.8, 7.3, and 10.2 respectively. The birth prevalence of Down syndrome fell by 80% from 1979-88 to 1994-99. Sensitivity of prenatal detection of congenital anomalies and TOPs were lower for isolated cases (only one malformation present in the fetus) than for multiple malformations in the same fetus. Sensitivity varied with the type of malformations : it was high for neural tube defect (79.7%) and urinary anomalies (54.8%) and low for congenital heart defects (25.3%) and for oral clefts (27.6%) **Conclusions** The introduction of routine prenatal diagnosis has resulted in a significant fall in the birth prevalence of congenital anomalies. However this fall varied with the types of congenital anomalies.

Prenatal detection of an interstitial deletion in 4p15 in a fetus with an increased nuchal skin fold measurement.

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The detection of a thickened fetal nuchal skin fold measurement is associated with an increased risk of common trisomies, cardiac defects and an expanding list of other genetic syndromes. We report a 19 year old para 1001 woman who was seen at 21 weeks gestation for a routine ultrasound evaluation that revealed fetal nuchal skin fold measurements of 6.5-8.9mm and a possible pericardial effusion. Following genetic counseling about the possible implications, amniocentesis was performed. A small, interstitial deletion in the short arm of one chromosome 4 was reported, 46,XX,del(4)(p15.2p16.1). Fluorescence in situ hybridization (FISH) using the Wolf-Hirschhorn syndrome probe showed clear signals at the tip of the p arm of both chromosomes 4, confirming that 4p16.3 was present. The maternal karyotype was normal. Paternal blood was unavailable for analysis. The patient elected to terminate the pregnancy and autopsy revealed no major malformations. Dysmorphic features noted included epicanthal folds, a prominent nasal bridge, micrognathia and low-set, posteriorly rotated ears. A series of BAC clones (ResGen Inc.) were prepared and used as FISH probes to refine the breakpoints in chromosome 4. The distal breakpoint lies within a 4Mb region between BAC RP11-81L15 (4p15.33) and BAC RP11-683L20 (4p15.31). The proximal breakpoint lies within a 6Mb region between BAC RP11-660M5 (p15.2) and BAC RP11-53F9 (p15.1).

Chromosome 4p15 deletions have been seen in children with developmental delay, dysmorphic facies, normal-to-accelerated growth and a possible connective tissue disorder. The prenatal finding of an increased nuchal skin fold measurement may be a marker for this particular chromosome abnormality. The pathogenesis of webbing of the neck, a reported feature of 4p deletions postnatally, may involve a gene located at 4p15.1 - 4p15.33. Precise phenotype-karyotype correlations, made possible by cytogenetic resolution using molecular techniques, are crucial in counseling parents when these rare, subtle structural abnormalities are encountered.

CLINICAL-GENETIC STUDY OF SIX FAMILIES WITH RECIPROCAL TRANSLOCATIONS. *R. Baez-Reyes, J.L. Saucedo-Hernandez, G. Razo-Aguilera.* Genetics Department. Instituto Nacional de Perinatologia, SSa. Mexico City.

The incidence of reciprocal translocations in the general population is 1 out of 500 persons (Jacobs,1977). These structural alterations can be detected in parents of children with congenital defects and/or mental retardation, in couples with recurrent spontaneous abortions or sterility. Germ cells produced by carriers of reciprocal translocations can often be unbalanced (Brandiff et al. 1986, Martin 1988), and this fact can lead to embrional and fetal developmental anomalies and limited intrauterine life or neonatal developmental anomalies; segregation of the chromosomes involved as well as the parent who is the carrier of the alteration have to be taken into account in order to figure out the risk in the offspring. Six different reciprocal translocations were observed over a period of two years in the Genetics of Reproduction and Prenatal Diagnosis offices of the Instituto Nacional de Perinatologia, the cytogenetic study indicated in periferal blood cells in 5 case of couples with recurrent gestational loss and in amniotic fluid cells in one case of a fetus with abnormal ultrasonography led to the following findings: t(2;5)mat.; t(3;7)mat.; t(5;7)pat.; t(1;18)mat.; t(1;14)mat and t(2;12)mat. All the couples were given genetic counseling but only four pregnancies were followed to the end, the babies were evaluated at birth and reevaluated periodically to date and even a pedigree studies were achieved. We concluded that the risk of the offspring from carriers of reciprocal translocations should be assessed one by one since it varies in each family depending on the chromosomes involved in the rearrangement, the breakpoints and the degree of genetic imbalance within the carriers germ cells. Prenatal diagnosis, assessment of the baby at birth, and followup checkups should be offered in case of a new pregnancie.

Prospective study of maternal serum screening for fetal chromosomal abnormalities: Clinical importance of false-positive rate. *F. ALVAREZ¹, M.L. SOTO QUINTANA¹, T. PADRON¹, A. ROJAS ATENCIO¹, K. URDANETA¹, A. MORALES MACHIN¹, M. PRIETO².* 1) UNIDAD DE GENETICA MEDICA, UNIVERSIDAD DEL ZULIA, MARACAIBO, ZULIA, VENEZUELA; 2) UNIVERSITY TULANE, NEW ORLEANS, LI.

Background: Maternal serum screening to identify fetal aneuploidy is now routinely offered during second trimester of pregnancy at developed countries. The purpose of this prospective study was to assess the value of maternal serum screening between 15 and 20 week of gestation to detect fetal aneuploidy and to determine the false positive rate (FPR) in a Venezuelan population. Study Design: Blood samples were collected on 1.062 women between 15 and 20 weeks gestation. Samples were assayed for alpha-fetoprotein (AFP), free beta human chorionic gonadotropin (b-hCG) and unconjugated estriol (uE3). Medians were established at each week from 200 normal, singleton pregnancies. Second trimester risk was calculated using the maternal age and different combinations of AFP, b-hCG and uE3. Screening results calculated by likelihood ratio to be equal to or greater than 1:270 were considered positive. If gestational age was confirmed by ultrasonography, genetic counseling and amniocentesis were offered. Results: Ten fetal chromosomal abnormalities were detected with maternal serum screening. Samples size does not allow correct detection rate estimation, but false positive rate (FPR) was found to be in 6,5%. This FPR has one clinical application. At a cut-off of 1:270, second trimester screening best results were obtained using combination of all three biochemical markers. Conclusions: These results confirm the efficacy of maternal serum screening for fetal chromosomal abnormalities with a low FPR. Measurement of AFP, b-hCG and uE3 is an effective prenatal screening test in Latinoamerican population.

Mosaicism for Monosomy 7 and Ring 7 detected through integrated prenatal screening for Down syndrome. *G.E. Johnson, E. Mak-Tam, D. Matias-Impey, H. Dorman, T. Huang, P.R. Wyatt, A.M. Summers.* Genetics Program, North York General Hospital and IODE Children's Centre, , Toronto, Ontario, Canada.

Integrated prenatal screening (IPS) combines first trimester nuchal translucency (NT) and pregnancy associated plasma protein-A (PAPP-A), with second trimester biochemical markers-alpha fetoprotein (AFP), unconjugated estriol (uE3), and human chorionic gonadotrophin (hCG) to screen for pregnancies associated with Down syndrome (DS), neural tube defects (NTD) and trisomy 18. It is expected to detect 85% of cases of DS at term with a false positive rate of 1.5%. Since July 1999, over 7,500 women have been screened using IPS in our centre. Here we report a case of mosaic ring chromosome 7 detected using IPS. A 35 year old Caucasian woman with a negative family history was screen positive for trisomy 18 (risk 1:50) on IPS. An amniocentesis was performed at 17 weeks gestation. Chromosome analysis on 10 metaphase cells showed 45,XY,-7. This karyotype was confirmed on a second amniocentesis; however, FISH analysis using the VYSIS CEP 7 probe on the second sample showed two signals in 90% of the interphase cells examined. The woman had a spontaneous abortion at 20 weeks. The fetus was nondysmorphic but preliminary pathology showed an interrupted aortic arch. Follow-up chromosome analysis of fetal tissue showed the presence of a ring chromosome 7 in 50% of cells in addition to the monosomy 7 cells. Re-examination of the two amniotic fluids did confirm a very low mosaicism for the ring 7 cell line. There are very few cases of mosaic ring 7 reported in the literature. Liveborn patients with the syndrome can have growth deficiency, craniofacial malformations, mental retardation, and pigmentary or vascular skin changes. Cardiac defects have not been reported previously. Our case is unique for 3 reasons: 1) the presence of a monosomy 7 cell line; 2) prenatal detection by IPS screen 3) the different cell line frequencies found in amniotic fluid, fetal tissue, interphase and metaphase analysis. This case illustrates the need for caution when interpreting mosaicism and the need for thorough follow up. These findings provide useful information for prenatal screening and genetic counseling.

Screening for aneuploidy using maternal blood spots in the first and second trimester. *S.B. Melancon¹, S. Demczuk¹, S. Zaor¹, M. Pelletier¹, D.A. Krantz², T.W. Hallahan², J.N. Macri².* 1) Procrea Genetic Services, Mont-Royal, PQ, Canada; 2) NTD Laboratories, Huntington Station, NY, USA.

Prenatal screening for Down syndrome (DS) and open neural tube defects (ONTD) using multiple maternal serum markers is now well established in the second trimester (T2) of pregnancy. Recent data suggest that maternal blood screening in the first trimester (T1) combined with an ultrasound (US) measurement of the fetal nuchal translucency (NT) would allow to reach around 90% sensitivity for DS at a 5% false positive rate. In collaboration with NTD Laboratories, PROCREA has been offering women from Eastern Canada, T1 and T2 maternal screening for DS and ONTD using dried blood spots on filter paper since 1999. NT measurements were selectively offered in T1 and US dating in T2. Before 13 6/7 weeks, PAPP-A and free b-hCG levels were coupled with fetal NT when available. Between 14 and 22 weeks, AFP and free b-hCG were assessed. Following positive screening results, couples were given the choice to follow up in a private or public clinical genetic setting. Until now, 20 640 pregnancies were monitored; 18 970 had T1 (92%) and 1 670 had T2. NT data were provided with 95,5% of T1 samples. There were 38 confirmed cases of DS, 36 in T1 and 2 in T2. In T1, 27/36 (75%) were at risk by blood markers alone, 24/33 (73%) by NT alone and 28/33 (85%) by combined blood/NT results. In one T2 case of DS, blood markers were negative. Using combined blood/NT data in T1, 32 other confirmed aneuploidies were found to be at risk; trisomy 18 in 8, trisomy 13 in 3, Turner syndrome in 10, triploidy in 8 and rare chromosomal anomalies in 3 more. 3 cases of ONTD were found at US in T1. One trisomy 18 and two ONTD were detected in T2. Our results agree with recent combined T1 screening data in DS and T18. In addition, we confirmed the high sensitivity of combined T1 screening in cases of Turner syndrome and in the other less common aneuploidies as well.

Prenatal Diagnosis of Chromosome Abnormalities in Saudi Arabia - A Report of 398 Cases M.A. Iqbal, M. Al-Yousuf, W. Kurdi. DPLM and OB/GYN, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia. M.A. Iqbal, M. Al-Yousuf, W. Kurdi. DPLM and OB/GYN, King Faisal Specialist Hospital&Research Center, Riyadh, Saudi Arabia.

Although extensive prenatal cytogenetic data is available elsewhere, very few reports are available for Arab countries. The present study describes retrospective data on fetal karyotyping performed on 398 prenatal samples between 1983-2001. The prenatal samples were of amniotic fluid (91%), chorionic villus samples (5%), fetal blood (4%). All of the samples were processed in the laboratory by routine cytogenetic techniques. Clinical indications: abnormal ultrasonographic findings (42.5%), AMA (29.1%), previous child with genetic abnormality (18.8%), carrier of balanced chromosome rearrangement (6.3%), and other indications (3.3%). The mean gestational age at amniocentesis was 24 weeks and at CVS was 12 weeks and 5 days, and cordocentesis was 27 weeks and 5 days. Out of 398 prenatal samples, successful fetal karyotyping was obtained in 385 cases (96.7%). Abnormal fetal karyotype was present in 42 cases (10.6%), and among these, numerical abnormalities were present in 21 cases (50%); autosomal (42.8%); sex chromosome (2.4%) and triploidy (4.8%). Structural chromosome abnormalities were seen in 17 out of 42 cases (40.5%) and mosaicism was observed in 4 cases (9.4%). Out of 169 fetuses with abnormal ultrasound findings, 20 fetuses (11.8%) had an abnormal karyotype, and among these, 17 cases (85%) showed numerical abnormalities. The remaining 15% had structural abnormalities. In the AMA group, 6 out of 116 (5.2%) fetal karyotypes were abnormal; interestingly, trisomy 21 was absent in this group. Out of 75 cases (18.8%) that had a previous child with chromosome abnormality, 70 (93.3%) were normal and 5 (6.7%) were abnormal. Balanced chromosome rearrangement was present in 25 cases (6.3%). Among these, 16 were normal (64%) and 9 (36%) were abnormal. There were 3 cases (0.8%) with a history of recurrent abortions; one of these was normal and 2 were abnormal. The results highlight the importance of fetal karyotyping in high-risk pregnancies. The data will be compared with that of other reported populations in the west.

Evaluation of Ductus Venosus Blood Flow as a Screening Marker for Down Syndrome in the First-Trimester. C. Rossi¹, E. Orlandi¹, F. Orlandi¹, D. Krantz², T. Hallahan², J. Macri². 1) Centro di Diagnosi Prenatale, Palermo, Italy; 2) NTD Laboratories, Huntington Sta, N.Y.

Ultrasound examinations in which crown-rump length, nuchal translucency and ductus venosus (DV) blood flow were evaluated were conducted on 729 first-trimester patients. After the first 78 patients the ductus venosus pulsatility index for the veins (DVPIV) was evaluated. Additionally, a maternal dried blood specimen was analyzed for free Beta human chorionic gonadotropin and Pregnancy Associated Plasma Protein-A. The combination of DV, nuchal translucency and biochemistry was successfully measured in 623 unaffected and 8 Down syndrome cases. The combination of DVPIV, nuchal translucency and biochemistry was successfully measured in 518 unaffected and 6 Down syndrome cases. Abnormal DV blood flow was observed in 26.2% of unaffected cases compared to 62.5% of Down syndrome cases ($P=.07$). The median DVPIV value of 1.15 in unaffected cases was significantly different than the 1.32 observed in Down syndrome cases ($P=.0278$). The false positive and Down syndrome detection rates using DV and DVPIV with maternal age were 15.2% and 75%, and 14.7% and 66.7%, respectively. Including DV into the risk assessment based on nuchal translucency and biochemistry increased the false positive rate from 5.5% (34 of 623) to 6.9% (43 of 623). Including DVPIV reduced the false positive rate from 5.0% (26 of 518) to 3.9% (20 of 518). Applying this approach only to patients whose risk based on nuchal translucency and biochemistry was increased, resulted in 14.7% (5 of 34) and 46.2% (12 of 26) cases being adjusted to within normal range with no loss in detection for DV and DVPIV, respectively. DVPIV, because of its quantitative nature appears to be a better Down syndrome screening marker than DV and should only be used as part of a multiple marker screening protocol. Additional studies are needed to determine if such a protocol should be applied to all patients or only to those with initial false positive results based on the nuchal translucency and biochemistry tests.

A diploid partial hydatidiform mole with microsatellite analysis. *J. Yankowitz, S.M. Zeng, W. Hansen.* Dept OB/GYN, Univ Iowa Hosps & Clinics, Iowa City, IA.

Partial hydatidiform moles (PHM) rarely complicates pregnancy, with an incidence of 0.001% to 0.01% of all pregnancies. PHM is thought to result from a triploid comprising two sets of paternal chromosomes and one set of maternal chromosomes in the fetus and placenta. This can be due to fertilization of a normal egg by two spermatozoa or duplication of a single spermatozoa. The pregnancy is characterized by fetal growth restriction and multiple congenital malformations with cystic changes in the placenta. There have been few reports that describe a normal diploid karyotype in PHM but it has recently been hypothesized that non-triploid PHM does not exist (Genest et al, *J Reprod Med* 2002;47:363-368).

Our patient was a 36 year old gravida 3 para 1. She underwent amniocentesis for maternal age which showed a 46,XX karyotype and normal AFAFP. Ultrasound showed that about 50% of the placenta was composed of confluent cysts. The fetus was normally grown with no anomalies. HCG was 44,257. After counseling about the possibility of partial mole, the patient underwent termination of pregnancy. Histologic evaluation of the placenta showed classic findings of partial mole.

We attempted to determine the genome ploidy and parental origin by analysis of polymorphic markers. Genomic DNA was extracted from molar and normal placenta, four types of fetal tissues (kidney, lung, muscle and eyeball) and parental peripheral blood. 52 polymorphic markers (1-2 markers for each chromosome) were chosen for genotyping. Each polymorphic locus was amplified by PCR and the PCR product was visualized on 6% polyacrylamide gel using silver-staining. Genotyping analysis from 38 informative polymorphic markers disclosed that the fetal tissues, normal placenta and molar tissue were all bi-allelic and of bi-parental origin (one paternal and one maternal). Karyotype of molar tissue also confirmed the original karyotype. Our results indicate that PHM can apparently take place with a diploid genome. These cases represent difficult counseling issues in the presence of normal anatomy and growth of the fetus.

Multiple fetal anomalies associated with subtle subtelomeric chromosomal rearrangements. *I.A. Glass¹, V.L. Souter², D.B. Chapman³, M. Raff¹, M.A. Parisi¹, K.E. Oheim⁴, C.M. Disteché³.* 1) Dept Pediatrics & Medicine, University of Washington, Seattle, WA; 2) Department of Medicine, University of Washington, Seattle; 3) Department of Pathology and Medicine, University of Washington, Seattle; 4) Department of Laboratories, Childrens Hospital and Regional Medical Center, Seattle.

We report two cases of multiple fetal anomalies detected by prenatal ultrasound and associated with subtle subtelomeric chromosomal rearrangements. The first case presented at 25 weeks of gestation with a posterior fossa cyst and ventriculomegaly. Karyotyping of amniocytes showed a subtle terminal abnormality of chromosome 6q. Thereafter, screening of all chromosomal subtelomeric regions using a panel of telomere-specific, fluorescence in situ hybridization (FISH) probes revealed an unbalanced reciprocal translocation between 6q and 17p [46,XX.ish der(6)t(6;17)(q25.3;p13) (TelVysion6q-;TelVysion17p+)]. The second case presented at 25 weeks of gestation with tetralogy of Fallot and at 34 weeks of gestation had additional ultrasound findings of an arachnoid cyst and intrauterine growth retardation. Karyotyping of peripheral blood was performed postnatally and appeared normal. However, a cryptic deletion of the subtelomeric region of the long arm of chromosome 14 was identified when the infants blood sample was used as a control for an oncology FISH probe (IGH-, Vysis Inc). Thereafter screening of all unique chromosomal subtelomeric regions using a panel of telomere-specific, fluorescence in situ hybridization (FISH) probes, revealed an unbalanced reciprocal translocation of chromosomes 14q and 20p [46,XY.ish der(14)t(14;20)(q32.3;p13)(IGH-,D14S308-,TelVysion20p+)mat]. These two cases add to a growing number of reports of cryptic subtelomeric chromosomal rearrangements associated with congenital anomalies. This is also the first report of multiple, simultaneous FISH screening of the subtelomeric regions in amniotic fluid and has demonstrated the technical feasibility of this technique in the prenatal period.

A novel Xp21 contiguous gene deletion associated with Duchenne muscular dystrophy, glycerol kinase deficiency, congenital adrenal hypoplasia and non-specific mental retardation. *S. Baert¹, V. Drouin-Garraud¹, F. Leturcq², P. Marret³, C. Forget⁴, T. Frebourg¹, P. Saugier-veber¹.* 1) Department of Genetics, CHU de Rouen, 76031 Rouen; 2) Laboratory of Biochemistry and Molecular Genetics, Hôpital Cochin, 75014 Paris; 3) Department of Neonatology, CHU de Rouen, 76031 Rouen; 4) Department of Pediatrics, CHG d'Elbeuf, 27000 Elbeuf, France.

The association of Duchenne muscular dystrophy (DMD), glycerol-kinase deficiency (GKD), and congenital adrenal hypoplasia (AHC) results from deletions of the Xp21 region. We report the case of a boy who presented hypotonia at birth and salt wasting at 14 days of life with normal 17 alpha-hydroxy-progesterone. Hypertriglyceridaemia, elevated serum glycerol and CPK (7197 U /L, normal range < 200 U/l) led us to suspect a complex glycerol-kinase deficiency. At 19 months of age, he has a developmental delay and short stature (<3rd percentile) without growth hormone deficiency and mild dysmorphic facial features. G-banding chromosomal analysis was normal. QMPSF (Quantitative Multiplex of Short Fluorescent Fragments) of the Xp21 region revealed that the index case and his mother carried a 3 Mb deletion removing exons 53-79 of *DMD* and the entire *GK*, *DAX-1* and *ILIRAPL1* genes. Partial deletions of the *ILIRAPL1* gene, involved in mental retardation, have already been described, but this report constitutes the first description of a contiguous Xp21 gene deletion removing entirely the *ILIRAPL1* gene. Detection of the *DMD* and *ILIRAPL1* genes deletion in this boy of 19 months of age represents a pre-symptomatic diagnosis of DMD and MR.

Markedly elevated maternal serum alpha-fetoprotein in a twin gestation with both twins heterozygous for Congenital Nephrosis (Finnish Type). *D.N. Saller Jr¹, C.J. Peterson², R.A. Mooney³, P. Martin⁴, S.R. Sanko², E.D. Warner², G. Schwartz⁵, J.A. Canick⁶, W.A. Hogge⁷, J.E. Haddow⁸.* 1) Dept OB/GYN, West Virginia Univ, Morgantown, WV; 2) Dept OB/GYN, Univ of Rochester, Rochester, NY; 3) Dept Pathology, Univ of Rochester, Rochester, NY; 4) Dept Biochemistry, Univ of Oulu, Oulu, Finland; 5) Dept Pediatrics, Univ of Rochester, Rochester, NY; 6) Dept Pathology, Brown Univ, Providence, RI; 7) Dept OB/GYN, Univ of Pittsburgh, Pittsburgh, PA; 8) Foundation for Blood Research, Scarborough, ME.

A 30 year old G3 P2002 female with a twin gestation had a maternal serum alpha-fetoprotein (AFP) at 16.7 weeks of 12.82 MoM. The family history and ultrasound were unremarkable. The amniotic fluid AFPs were 20.65 MoM and 25.01 MoM and the acetyl cholinesterases were both negative. Both karyotypes were 46,XX. DNA testing for Congenital Nephrosis (Finnish Type) (CNF), at the University of Oulu (Finland), indicated both parents were heterozygotes. The father carried a stop codon in exon 8 (previously not identified, but similar stop codons have been found in affected individuals). The mother carried a promoter sequence deletion (previously identified in an affected individual). Analysis of the fetal DNA indicated that both twins were heterozygotes, each carrying the paternal sequence variation only. The amniotic fluid AFP, however suggested an affected pregnancy. The patient was extensively counseled and opted to continue the pregnancy. The remainder of the pregnancy (including serial ultrasounds) was unremarkable. She delivered at 38 weeks by spontaneous vaginal delivery. Initial neonatal urines showed +1 and trace protein. Both twins had trace proteinuria at hospital discharge and have had no proteinuria (negative) on serial urinalysis through 12 months of life. Both twins are healthy and developing appropriately at age 19 months. This case appears to clinically confirm that a single (heterozygote) CNF mutation may affect fetal renal function in the second trimester, causing markedly elevated serum and amniotic fluid AFP, as suggested by Patrakka et al (Lancet 2002;359;1575-7) on terminated pregnancies. Therefore all suspected cases of CNF should be confirmed by molecular studies.

Three consecutive triploidy pregnancies in a 35-year-old woman: genetic predisposition? *B. Huang¹, L. Prensky², M. Thangavelu¹, S. Wang³.* 1) Genzyme Genetics, Orange, CA; 2) California Pacific Medical Center, San Francisco; 3) Harbor-UCLA Medical Center, Torrance, CA.

Triploidy accounts for almost 17% of abortus with chromosome abnormalities. Several mechanisms have been proposed for triploidy including: 1) dispermy; 2) inclusion of polar body during meiosis I or meiosis II and 3) paternal meiosis error resulting in diploid sperms. However, the genetic or environmental factors contributing to the occurrence of triploidy remain unknown. We report a 35-year-old G3P0TAB1SAB2 woman with three consecutive triploidy pregnancies. The patient has a family history of multiple miscarriages (8 miscarriages in paternal grandmother) and infertility (paternal great aunt). She had her first pregnancy at age 31. Ultrasound examination detected multiple abnormalites and the pregnancy was terminated at 16 weeks of gestation. Chromosome analysis of the product of conception revealed a 69,XXX karyotype. The following 2 pregnancies occurred when she was 34 and 35 years of age, respectively. Both pregnancies were miscarried at 8 weeks of gestation. Chromosome analysis of the products of conceptions showed 69,XXY karyotype for both of the pregnancies. There is no other detectable pregnancies. In an attempt to understand the mechanism of repeated triploidy in this couple, microsatellite analysis with DNA markers from multiple chromosomes was performed on the cells from the last pregnancy. The results showed that the fetus inherited two alleles from the mother and one allele from the father, consistent with inclusion of the polar body during maternal meiosis. The information gained from the study will help the family to make informed decisions regarding future reproduction options, particularly in the use of assisted reproductive techniques. In addition, the patient's strong family history of reproductive difficulties and her pregnancy history suggests a possible genetic predisposition to triploidy in this family.

Frequency of congenital defects in newborns of women with diabetes during pregnancy. *M. Aguinaga, I. Llano, C. Hernandez, JM. Garza, DG. Mayen-Molina, R. Garcia-Cavazos.* Departamento de Genetica. Instituto Nacional Perinatologia, Mexico DF, Mexico.

Skipper in 1933 was the first to notice that maternal diabetes was associated with an increased occurrence of congenital malformations. Congenital defects occur 2 to 4 times more frequent in newborns of diabetic women and include cardiopathy, neural tube defects, holoprosencephaly, caudal regression syndrome, renal, gastrointestinal, craneofacial and multiple musculoskeletal abnormalities. The data was collected from the Registry of congenital birth defects of the Instituto Nacional de Perinatologia in Mexico City during the period of 1998 to 2001. Of 504 newborns with congenital defects, we selected only patients with diabetic mothers. There were a total of 70 cases which represents 13.8% of our population. Of these, we detected 27 major congenital defects of which 11 were craneofacial, 5 musculoskeletal, 7 abnormalities of central nervous system, 2 renal and 2 other alterations. Thirty-three minor congenital defects: 21 craneofacial, 7 musculoskeletal and 5 other abnormalities. Ten patients had multiple congenital defects. In our population, newborns of diabetic women account for an important number of patients with congenital defects.

Klippel-Trenaunay Syndrome presenting as possible Amniotic Band Syndrome. *P.L. Devers¹, A.F. Wagner¹, M.C. Treadwell², R. Romero³, G. Conoscenti³, A. Johnson¹.* 1) Div. of Reproductive Genetics, Dept. of Ob/Gyn, Hutzel Hospital, Wayne State University, Detroit, MI; 2) Div. of Maternal-Fetal Medicine, Dept. of Ob/Gyn, Hutzel Hospital, Wayne State University, Detroit, MI; 3) Perinatal Research Branch, NICHD/NIH, Bethesda, MD.

Background: Klippel-Trenaunay syndrome (KTS) is a rare, congenital disorder consisting of combined vascular malformations, varicosities of unusual distribution, and limb enlargement. The antenatal diagnosis of KTS by 2D ultrasound has been well documented. We describe a case of prenatally diagnosed hemihypertrophy suspected to be secondary to amniotic band syndrome (ABS), which was ultimately determined to be KTS.

Case: LM, a 24 y/o G5P2-0-2-2 AA female, presented at 18 2/7 weeks gestation after targeted fetal ultrasound revealed hemihypertrophy of the lt. leg. Both 2D and 3D ultrasound detected thin sheets of amnion extending around the upper and lower leg. Increased vascular resistance was noted in the lt. femoral artery. The remainder of the fetal anatomy appeared normal. The findings were felt to be highly suggestive of vascular obstruction secondary to ABS. Fetoscopy was performed at 21 3/7 weeks gestation for possible excision of the constrictive bands. No identifiable bands were detected; however, multiple bullous lesions and "port-wine" nevi were seen on the edematous leg. Delivery was at 38 2/7 weeks. The lt. leg was noted to have non-pitting edema from thigh to toe with a hemangiomatous lesion extending from the gluteal region to the lateral aspect of the thigh and calf. The diagnosis of KTS was confirmed with the ultrasonic finding of a cavernous hemangioma.

Discussion: The differential diagnosis for prenatally detected asymmetric limb growth would include multiple Mendelian disorders or vascular obstruction secondary to venous thrombosis or ABS. Fetoscopic intervention proved beneficial in eliminating the suggestion of the latter and detection of the dermatologic lesions consistent with KTS.

The role of genetic counseling in assisted reproductive technologies. *A.B. Ilagan¹, T. Hassold¹, P.A. Hunt¹, R. LoretdedeMola², A. Matthews¹.* 1) Human Genetics, Case Western Reserve Univ., Cleveland, OH; 2) Dept. of Reproductive Biology, University Hospitals, Cleveland, OH.

Infertility remains a major health problem affecting 6.1 million people of reproductive age in the United States. Treatment includes a variety of laboratory techniques known collectively as assisted reproductive technologies (ART). Although thousands of children have been conceived by ART since its inception in the late 1970s, associated reproductive risks are continually being assessed and defined. These risks, along with the fact that there are a number of known genetic causes of infertility are compelling reasons why genetic counseling should be an essential part of ART treatment. However, there is no literature that defines the current role of genetic counseling in ART. Therefore, the purpose of this study was to describe how genetic counseling services are currently utilized in reproductive medicine. A total of 406 ART clinics across the United States were sent questionnaires regarding this study. 92 ART clinics completed the questionnaire. Results showed that genetic counselors were employed at 21 clinics. Eleven clinics employed full-time counselors, while counselors worked part-time at 10 clinics. Of the remaining 71 clinics, 67 referred patients to an off-site genetic counselor. Also included in the questionnaire were three vignettes presenting scenarios that involved specific genetic issues, after which participants were asked to describe their current protocol for each situation. Only 50% of respondents indicated that genetic counseling would be included in their protocol for patients with appropriate indications for referral. Given these results, although it was encouraging that genetic counseling was identified as a resource by 96% of participating clinics, it appears that genetic counseling is still underutilized by reproductive specialists in ART. By increasing awareness about the many different roles genetic counseling plays in the ART community, reproductive care would be enhanced for many individuals undergoing ART treatment with underlying genetic causes for their infertility.

b thalassemia prevention program in Iran. *R.R. Karimi-Nejad¹, S. Teimourian¹, F. Sahebjam¹, Y. Shafaghati², S. Hoseini¹, M. Babamohamadi¹, N. Almadani¹, H. Karimi-Nejad¹, H. Najmabadi^{1,2}.* 1) Karimi-Nejad Genetics Ctr, Tehran, Iran; 2) University of Social And Welfare And Rehabilitation sciences.

The most common preventable hereditary disease in Iran is Beta-Thalassemia. The carrier frequency in some regions of the country, is as high as 1 in every 4 individuals. Preventive approach for Beta-Thalassemia started a decade ago as a self-motivated and regulated social need in the form of prenatal diagnosis. Although Iran is a religious country and therapeutic abortions were considered to be unacceptable and against the beliefs of Islam, based on the needs of the society to prevent the widespread of Beta-Thalassemia prevention program was established by the authorities. Iranian geneticists decided to hold a meeting with religious officials to discuss the role of prenatal diagnosis, particularly for B-Thalassemia. It was not until a few years ago when religious leaders accepted that the spread of B-Thalassemia needed to be controlled and issued permission to perform therapeutic abortions up to 16 weeks of gestation for Beta-Thalassemia affected individuals. Simultaneously, the government began to set up an involve systematic approaches for the control and prevention of Beta-Thalassemia. Over the past few years, Iran has also developed a coverage system that forces insurance companies to cover these types of medical expenses; this has been incredibly beneficial in accelerating the prevention program. The government in order to help the prenatal diagnostic program has also approved two national reference laboratories. These reference laboratories are responsible for providing training problem solving, quality control, and quality assurance for themselves and referral laboratories. Today in Iran, effort of the past decade is showing its effect on the society. We are expecting over 1000 prenatal diagnosis to be done this year. Our system is highly efficient and compatible for the neighboring countries. It can be used as a functional adaptive system for any other Islamic countries. In this study we are reporting a total of 2576 chromosome from 2462 individuals of 788 families during 12 years, which has resulted in 541 prenatal diagnosis.

Quantitative Analysis of Fetal DNA in Maternal Serum Before and After Delivery. *M. Hyodo, O. Samura, N. Miharuru, K. Ohama.* Dept.OB/GYN, Hiroshima univ.sch.medicine Japan.

Objective: It has been demonstrated that fetal DNA can be detected in maternal blood during pregnancy and that this DNA can persist for many years after delivery as intact fetal cells and possibly as cell-free fetal DNA. However, it has not been demonstrated how the concentration of fetal DNA in maternal serum change practically before and after delivery, and how long this DNA remains in the maternal circulation. This study was performed to determine the change in the concentration of fetal DNA in maternal serum before and after delivery. **Materials and methods:** The study cases consisted of 20 women carrying male fetuses and 4 women with female fetuses were included as negative controls. Quantitative PCR assay of the sex determining region on the Y chromosome (SRY) was used as a maker of male fetal DNA. We collected peripheral blood from these women at three time points; immediately before delivery, immediately after delivery and one day after delivery. We extracted DNA from 1.2mL of each serum sample and concentrated it into 70L of water. We performed real-time PCR for the SRY gene sequence to detect male fetal DNA in these samples. **Results:** No PCR amplification products of the SRY sequence were detected in the controls. In 20 women carrying male fetuses, the concentration of the SRY sequence in maternal serum before delivery (mean; 85.72copies/mL, range; 16.42-246.96copies/mL) was significantly higher than that after delivery (mean; 64.24copies/mL, range; 2.92-202.53copies/mL)($p=0.03$). One day after delivery, 9 of 20 women with male fetuses had no fetal DNA in their serum, and the mean concentration of the SRY sequence in the other 11 maternal serum was 6.13copies/mL(range; 0.19-15.97copies/mL). **Conclusions:** Fetal DNA was detected in maternal serum before and after delivery, and fetal DNA deceased rapidly by one day after delivery.

Immunofluorescence following FISH: Application to microchimerism studies. *H.P. Stroh, K. Khosrotehrani, D.W. Bianchi, K.L. Johnson.* Division of Genetics, Department of Pediatrics, Tufts-New England Medical Center, Boston, MA.

Objective: Fetal cell microchimerism has been demonstrated in a number of diseases, predominantly autoimmune in nature. The fetal origin of the microchimeric cells has been confirmed by genetic analysis (i.e. PCR and/or FISH). To better understand the functional characteristics of the persistent microchimeric cells, we assessed the feasibility of combining molecular cytogenetic and immunofluorescence methods on archived tissue specimens.

Methods: Paraffin-embedded tissue sections (5 μ M) were subjected to FISH analysis using X and Y chromosome-specific probes, each in a unique fluorescent color. For immunofluorescence analysis, two primary monoclonal antibodies were used: 581 (anti-CD34) and AE1/AE3 (anti-cytokeratin). After performing FISH and without any other pretreatment, slides were washed in PBS, incubated with the primary antibody for one hour (AE1/AE3) or overnight (581), washed and subjected to the detection system. Fluorescent microscopic observation of both histochemical and cytogenetic signals was then performed.

Results: Cytokeratin detection proved successful and stained epidermis in skin and hepatocytes in liver samples, without compromising the FISH signals. CD34 detection failed on spleen, liver and skin samples. Two detection systems were used: a Texas-Red labeled avidin-biotin and a phycoerythrin (PE) goat anti-mouse immunoglobulin antibody. The avidin-biotin detection system resulted in endogenous avidin binding activity with false positive staining. The PE-labeled secondary antibody eliminated this problem.

Conclusions: We have successfully combined immunofluorescence and FISH, using the AE1/AE3 anticytokeratin antibody. In our experience surface markers like anti-CD34 seem to be less effective with this protocol. We believe that internal proteins are best suited for this combined method, although each antibody should be tested empirically.

Recurrent hydatidiform mole: Further mapping and candidate gene analysis on chromosome 19q13.4. P. Panichkul, A. Rajkovic, C.D. Kashork, L.G. Shaffer, I.B. Van den Veyver. Baylor College of Medicine, Houston, TX.

Previous studies support that familial recurrent hydatidiform moles (HMs) have a diploid biparental genomic contribution; affected women carry an autosomal recessive mutation that maps to a region on chromosome 19q13.4 containing imprinted genes. We hypothesized that an oocyte-specific imprinting defect in these women causes their conceptus to behave as a HM with excess paternal genome. Judson, et al. (Nature 2002,416:539-42) found evidence for such an imprinting defect in a patient with 6 recurrent HM. Because familial HM are rare, we hypothesized that patients with sporadic recurrent HM have the same defect and can be studied for additional homozygosity mapping to narrow the critical region. We performed haplotype analysis on two such patients (Mo1 and Mo2) with densely-spaced polymorphic markers in and around the candidate region. Mo1 was homozygous for all markers; Mo2 was homozygous for a smaller interval flanked by D19S418 and D19S404. PCR amplification and direct sequencing of coding exons of *ZIM3* (Zinc-finger gene 3), a paternally imprinted gene and *ZNF264*, a maternally imprinted gene within this region did not reveal mutations in Mo1 and Mo2. A newly identified oocyte-specific gene, *RFPL4* (encoding Ret finger protein-like 4), localized in the candidate region, was also not mutated in Mo1, Mo2. Southern analysis of genomic DNA from Mo1 and Mo2, digested with 6 restriction enzymes and probed with coding exons of these genes did not detect genomic rearrangements. We excluded that a larger deletion was responsible for the apparent marker homozygosity by performing FISH on metaphase spreads from lymphoblasts of patient Mo2 with a BAC clone that maps within the region (no cell lines on other patients were available).

Our findings support that isolated and familial recurrent HM have the same etiology, but additional patients will be studied to confirm this and to narrow the critical region. We further conclude that *ZIM3*, *ZNF264* and *RFPL4* are not mutated in these 2 patients with recurrent HM.

Analysis of imprinted genes on 11p15.5 associated with intrauterine growth. *S. Apostolidou*¹, *A. Williams*¹, *K. O'Donoghue*¹, *Z. Montamedi*¹, *P. Stanier*¹, *P. Loughna*², *G.E. Moore*¹. 1) Maternal and Fetal Medicine, IRDB, Imperial College, Ducane Road, London W12 0NN, United Kingdom; 2) City Hospital, Nottingham NG5 1PB, United Kingdom.

Intrauterine growth restriction (IUGR) contributes greatly to perinatal and childhood morbidity and mortality. IUGR fetuses show ultrasound evidence of slowed growth and are at increased risk of intrauterine hypoxia, acidosis and death. In later life they also have an increased risk of hypertension, glucose intolerance and death from coronary heart disease. The aetiology of IUGR is multifactorial with genetic and environmental factors playing a role. In both mouse and human, disruption of normal imprinting mechanisms has been associated with abnormal growth phenotypes, implicating imprinted genes in the control of size at birth. Chromosome 11p15.5 contains a number of paternally and maternally imprinted genes; *IGF2*, *p57^{KIP2}*, *TSSC3*, *TSSC5*, *H19*, *KCNQ1*, *RNH*. Disruption of *IGF2* imprinting has been found in Beckwith-Wiedemann syndrome and tumours, whereas in transgenic mice deficiency of *Igf2* leads to 60% growth restriction. Quantitative expression analysis of *IGF2* and other imprinted genes on 11p15.5 has been carried out on >200 samples of placenta from consecutive births to compare with fetal growth. Additionally, upstream of *IGF2* is insulin and both genes are regulated by a variable number tandem repeat, the *INSVNTR*. The repeat number or class size and the parent-of-origin of the *INSVNTR* are known to affect both the expression of insulin and *IGF2* in fetal tissues. Smaller repeats increase the risk of type I diabetes and longer repeats are associated with type II diabetes and polycystic ovary syndrome. The same 200 births including parental DNAs (trios) have also been genotyped for the *INSVNTR* class size and parent-of-origin inheritance. Correlations will be drawn between expression levels of the imprinted genes, parent-of-origin, size of the *INSVNTR* and fetal growth in utero.

The genetic contribution to male subfertility: a clinical and molecular genetic study. *C.A. Gardiner¹, K.J. Flintoff², G.R. Taylor², V. Sharma³, A.J. Rutherford⁴, A.H. Balen⁴, C.E. Chu⁵.* 1) Dept Clinical Genetics Service, Nottingham City Hosp, Nottingham, England; 2) Yorkshire Regional DNA Laboratory, Leeds. UK; 3) Assisted Conception Unit, St James University Hospital, Leeds. UK; 4) Assisted Conception Unit, The General Infirmary at Leeds, Leeds. UK; 5) Yorkshire Regional Genetics Service, Leeds. UK.

Approximately 1 in 10 couples seek medical help for subfertility and in about 25% of these cases there is solely male factor subfertility. Epidemiological studies have shown that some male subfertility is familial. Approximately 14% of azoospermic men and 5% of oligozoospermic men have a chromosome abnormality and about 10% of men with azoospermia and 5% of men with oligozoospermia have a Y-chromosome microdeletion. The mean androgen receptor (AR) CAG repeat length has been reported to be significantly longer in male subfertile patients than in fertile controls. Ninety-three men with azoospermia or oligozoospermia and fifty fertile controls agreed to take part in a study, which involved obtaining a three generation pedigree and molecular genetic analysis for Y-chromosome microdeletions and the size of the CAG repeat length in the AR. One man was identified as having a Y-chromosome re-arrangement and one man was identified as having a Y-chromosome microdeletion. There was no statistical difference in the size of the CAG repeat length between the patient and the fertile control population. Five percent of the study population had one first degree relative with subfertility and none of these men had a chromosome abnormality or a Y-chromosome microdeletion. Other genes or environmental factors are involved in male subfertility and family history appears to be currently the only way to identify this group of men. Identification of these families could allow mapping of genes involved in male subfertility or identification of environmental factors that may affect male fertility.

Quantification of gene expression in testicular tissue specimens of idiopathically infertile males. *R. LAVERY¹, J.A Houghton³, A. Nolan², M. Glennon¹, D. Egan³, M. Maher¹.* 1) National Diagnostics Centre, BioResearch Ireland, National University of Ireland, Galway; 2) Fertility Unit, University College Hospital, Galway; 3) Microbiology Department, National University of Ireland, Galway.

Quantitative real-time PCR is increasingly used to quantify copy numbers of nucleic acids for clinical applications. In this study the LightCycler real-time PCR system was used to quantify gene expression levels of selected genes associated with spermatogenesis in a cohort of controls (n=6) and idiopathically infertile males (n=15). The application of quantitative real-time reverse-transcriptase-PCR (RT-PCR) technology for measuring the expression of a number of candidate Y-chromosome genes together with ZNF genes, Protamine-2 and b-actin, the housekeeping gene, was investigated. RNA was extracted from testicular biopsy specimens using Trizol and DNase treated to remove contaminating DNA. LightCycler reactions were prepared using 0.5mg of DNase treated testicular RNA. Quantification of mRNA was performed by comparison to a standard curve generated following RT-PCR amplification and purification of 103,106 and 108 molecules of each gene of interest using the LightCycler - RNA amplification kit SYBR Green 1 (Roche Molecular Biochemicals). To date, Protamine-2 transcribed post-meiotically in the round spermatid stage of spermatogenesis and the ZNF230 gene have been analysed. The Protamine-2 gene was expressed in controls and clinical samples positive for the presence of sperm at biopsy giving mean Ct values of 21.905 and 24.23 respectively. A Ct value of 38.7, below the value of the negative control Ct value, was observed in negative biopsies therefore confirming findings on examination of the biopsy. The newly identified ZNF230 gene (Zhang et al, 2001), previously reported to be undetectable in the testes of azoospermic patients was also analysed by real time RT-PCR. Controls and azoospermic patients had mean Ct values of 18.28 and 20.38 respectively. Histological analysis of testicular biopsy material from patients is also being preformed with a view to suggesting a genotype/ phenotype correlation for the patients.

Identification of the Simpson-Golabi-Behmel Syndrome type 2 gene candidates on chromosome Xp22. *B. Xu*¹, *R.P. Hart*², *Z. Zhang*³, *R. Weksberg*⁴, *L.M. Brzustowicz*^{1,5}. 1) Department of genetics, Rutgers university, Piscataway, NJ; 2) Department of Cell Biology and Neuroscience, Rutgers University Piscataway, NJ; 3) Department of Microbiology and Immunology, Temple University, Philadelphia, PA; 4) Departments of Paediatrics and Genetics, The Hospital for Sick Children and Departments of Paediatrics and Molecular and Genetics, University of Toronto, Canada; 5) Department of Psychiatry, UMDNJ, New Jersey Medical School, Newark, NJ.

The gene for Simpson-Golabi-Behmel syndrome (SGBS) Type 2 has been mapped to an approximately 6 Mb region on chromosome Xp22. This area is predicted to contain 100 to 200 genes, a daunting number to individually mutation screen. The Human Genome Project has sequenced approximately 90% of this region. We have utilized bioinformatics methods to build a catalog of gene structures in this interval. To utilize information generated by the Human Genome Project, the public genomic annotation project, OMIM, and the EST database, a pipe application has been developed to parse genomic annotation data, process EST assemblies and spliced EST/genomic alignments, and simultaneously pipe all data into Artemis, a Java visualization program. This approach has identified 234 candidate sequences in this region. These represent 46 known genes or sequences with strong experimental evidence and 188 EST assembled contigs. These candidates were then used for initial transcript profile screening. Clinic and knock-out mouse studies have demonstrated the importance of the IGF-2 pathway in the overgrowth syndromes. The human HepG2 cell line was employed as a model cell system and utilized to assess the effects of manipulation of the IGF-2 pathway on level of expression of the 234 candidates. Gene expression profiles were established by real-time PCR. Sixty-five of the candidates were detectably expressed in the HepG2 cell line. Among them, 13 genes (4 known genes and 9 EST assembled contigs) showed reproducible expression changes after exposure to IGF-2. These genes are potential candidates for SGBS Type 2, and represent a manageable number to screen for causative mutations. Further study of these candidates will be conducted on samples from a patient with SGBS Type 2 and normal controls.

Preliminary estimate for the detection rate of 45,X pregnancies using the second trimester maternal serum triple test. *P.A. Benn¹, J. Ying²*. 1) Div Genetics, Dept Pediatrics, Univ Connecticut Health Ctr, Farmington, CT; 2) Dept Statistics, Univ Connecticut, Storrs, CT.

Objective: To estimate the detection rate for 45,X pregnancies through second trimester maternal serum Down syndrome and trisomy 18 screening. Also, to evaluate a screening protocol specifically designed to identify 45,X pregnancies. **Methods:** 22 cases of 45,X were ascertained through a cytogenetics database and an additional 51 cases were identified through publications. Screening results (maternal serum alpha-fetoprotein, human chorionic gonadotropin, and uncojugated estriol) were reviewed for cases with fetal hydrops, cystic hygroma alone, and, no evidence of edema. For modeling purposes, 45,X cases were categorized on the basis of their hCG values (Type A >1.0 MoM and Type B <1.0 MoM). Means, standard deviations, and correlation coefficients were calculated and detection rates and false-positive rates established through computer simulations. Second trimester screening cut-offs were 1:270 for Down syndrome and 45,X Type A pregnancies and 1:100 for trisomy 18 and 45,X Type B pregnancies. The 45,X screening protocol was based on modification of a second trimester maternal age-specific prevalence curve for 45,X. Detection rates for Type A and Type B cases were combined and the overall efficacy evaluated for the 1999 U.S. pregnancy population. **Results:** Approximately 49% of all 45,X pregnancies should be identifiable through Down syndrome and trisomy 18 triple testing. The detection rate for cases with hydrops or cystic hygroma was 52% and, when edema was absent, 41%. If offered in conjunction with Down syndrome and trisomy 18 screening, the 45,X screening protocol could identify an estimated 15% more of the affected pregnancies with an incremental gain of 0.9% in the false-positive rate. For women aged <35 years, the incremental gain in detection and false-positive rate was 16% and 1% respectively, while for women aged ≥35 the gain in the detection rate and false-positive was 7% and 0.3% respectively. **Conclusion:** A worthwhile screening algorithm for 45,X could be developed that would primarily benefit younger women.

Accuracy of expected risk of trisomy 18 using the second trimester triple test. *C. Meier, T. Huang, PR. Wyatt, AM. Summers.* Dept Genetics, North York General Hosp, Toronto, ON, Canada.

Since the mid-1990s many centres have used an individual risk method for calculating trisomy 18 risk (Palomaki 1995). Although this method had been widely used, the accuracy of the individual risk has not been well evaluated. Using data from a large provincial programme, we tested the accuracy of estimated risk of trisomy 18 syndrome. The study was based on 382,568 women screened in the Ontario maternal serum screening programme between October 1993 and September 2000. Ninety-four percent of them had known pregnancy outcomes including 108 affected pregnancies. 92,874 were screened using risk cut-off level method (3 1 in 100). The remaining women were screened using a fixed analyte cut-off method. The expected risks for Trisomy 18 for the former group were calculated for this study using AFP Expert (Benetech, ON, Canada) and by applying published population parameters. The age specific prevalence for trisomy 18 was estimated as one tenth of the Down syndrome prevalence for the same age.

Women screened were ranked according to their individual expected risk of Trisomy 18 syndrome. They were divided into nine groups, each group containing 5 to 50 cases of Trisomy 18 pregnancies. The mean expected term risks of having an affected pregnancy were calculated for each group. The risks were then compared with the observed risks (prevalence) of that particular group. Since it is estimated that 70% of Trisomy 18 pregnancies will abort spontaneously subsequent to midtrimester, cases with positive screening results and diagnosed prenatally were multiplied by 0.3 to allow for the spontaneous fetal losses. Close agreement between the mean expected risks and the observed prevalence was seen across lower risk groups, but women identified as having high-risk (by calculation) pregnancies had an actual prevalence that was somewhat less extreme. To our knowledge, this is the first study looking at this question. Our results show the expected risks of Trisomy 18 syndrome assigned to individual women were on average, close to the observed risks across all the risk ranges.

The levels of maternal serum markers for Down syndrome screening in Aboriginal Canadian women. *T. Huang¹, AM. Summers¹, PR. Wyatt¹, C. Meier¹, G. Côté²*. 1) Genetics program, North York Hospital, Toronto, Ontario, Canada; 2) Sudbury Regional Hospital, Sudbury, Ontario, Canada.

In second trimester maternal serum screening (MSS) for Down syndrome, a woman's risks of having a term Down syndrome pregnancy is calculated by combining her age specific risk with her serum marker levels. Previous studies have reported ethnic differences in levels of serum markers, with the major differences seen between Black and Caucasian women. We conducted a study to investigate whether the levels of MSAFP, MShCG, and MSuE3 in Aboriginal women are different from those of Caucasian women. The study was conducted in 7717 Caucasian and 413 Aboriginal women (mostly Ojibway, Oji-Cree and Cree) with singleton pregnancies screened in the Ontario MSS programme between October 1993 and September 1998. Women with multiple pregnancies, pregnancies affected with Down syndrome, neural tube defects, or Trisomy 18, those with insulin-dependent diabetes mellitus and those without weight recorded were excluded from the study. No differences in the levels of maternal serum alpha-fetoprotein and total human chorionic gonadotrophin were observed between the two groups. Maternal serum unconjugated estriol was 15% higher in Aboriginal women. Similar results were seen even after controlling for the influences of age and parity on serum markers. Since Aboriginal women make up only a small proportion of women screened, correcting the level of uE3 for this group will have little effect on screening performance for the whole of Ontario. However, if these results are confirmed by further study, individual centres may consider making this correction, so that similar screening performance can be achieved in Caucasian and Aboriginal women.

Maternal weight impact on maternal triple-marker serum concentrations in a Venezuelan population. *M.L. Hernández, F. Álvarez-Nava, M. Soto, T. Padrón, M.C. Martínez.* Unidad de Genética Médica, Universidad del Zulia, Maracaibo, Venezuela.

Background: Maternal serum screening by means of alpha-fetoprotein (AFP), free b-subunit of chorionic gonadotrophin (b-hCG) and unconjugated estriol (uE3) is broadly consolidated at clinical level allowing to reach a detection rate of 60-70% of syndrome Down fetus. It is known that variables modifying these values exist. The objective of this study is to determine the influence of maternal weight, presence of diabetes mellitus, maternal smoking habits, gravidity, parity and fetal gender on the level of maternal serum markers used in second trimester screening.

Study Design: Maternal serum AFP, b-hCG and uE3 concentrations had been measured in 1062 singleton unaffected pregnancies from 15 to 20 week in two centres of Maracaibo, Venezuela. Information on maternal weight, cigarette smoking, maternal insulin-independent diabetes mellitus, gravidity and parity was available in all pregnancy, fetal gender was only available in 445 amniocentesis was carried. All results were expressed as multiple of the gestation specific median (MoM) values.

Results: All three markers showed a statistically significant negative association with maternal weight ($p < 0,05$) and subsequent analyses MoM values were adjusted using regression analyses. The MoM three serum markers levels in primigravidas was significantly greater than that in non-primigravidas but when it was weight adjusted this differences was not present. Similar results were found for parity. No difference was found in the serum level of all markers in female or male pregnancy.

Conclusions: Maternal weight is most important second trimester screening covariable. Our results emphasize the need for weight correction in serum screening for fetal aneuploidies.

Role of Morbid Obesity and Consanguinity in Maternal Serum Alpha-fetoprotein Screening. *S.B. Reddy¹, S. Reddy², S.S. Reddy³, I. Khanfer³, A.K. Roy³.* 1) Alpha Feto-Protein , Natl Medical Diagnostic Lab, Southfield, MI; 2) Peoples Clinic, Detroit, MI; 3) St. Joseph Medical Center, Detroit, MI.

We have screened African-American and Asian Indian pregnant women for prenatal screening of Neural tube defects and chromosomal defects employing Triple test (MSAFP, MShCG and MSuE3). Our Maternal Serum Alpha-feto Protein (MSAFP) values are adjusted for maternal weight, race and Insulin dependent Diabetes Mellitus (IDDM). In our investigation 97 morbidly obese pregnant women in African-American population weighed 390-402 lbs and 58 in Asian Indian population 270-375 lbs. Out of these 58 morbidly obese Indian pregnant women 22 marriages are consanguineous. They are married either to their first or second cousin or maternal uncle. There have been several reports in the past to show the relationship between maternal weight and MSAFP in different ethnic groups. It has been established that a diluting effect of larger plasma volume is attributed to the lower median values in heavier pregnant women. Thus, heavier women generally have lower concentrations of circulating AFP. That is the reason the weight adjustments are to be made to obtain the precise prenatal risks. The analysis of the outcome data for Neural tube and chromosomal defects we have not found any difference between these two populations of pregnant women. However we found a significant proportion of chromosomal defects was exhibited in the Asian Indian pregnant women in excess of 350lbs with consanguineous marriage. Data and statistics with plausible mechanisms including consanguinity coupled with morbid obesity will be presented.

Antenatal manifestations of Smith-Lemli-Opitz syndrome. A. Goldenberg¹, C. Wolf², F. Chevy², A. Benachi³, Y. Dumez³, A. Munnich¹, V. Cormier-Daire¹. 1) Department of Medical Genetics, Necker-Enfant-Malades Hospital, Paris, France; 2) Laboratory of Mass Spectrometry, Saint-Antoine Hospital, Paris, France; 3) Department of Obstetrics, Necker-Enfants-Malades Hospital, Paris, France.

Smith-Lemli-Opitz syndrome (SLO) is an autosomal recessive multiple malformation and mental retardation syndrome ascribed to a 7-dehydrocholesterol reductase deficiency, usually diagnosed in the early postnatal period. Prenatal diagnosis based on dehydrocholesterol measurement on chorion villi or amniotic fluid is highly reliable. Reviewing a series of 30 cases of SLO, we have addressed the question of the antenatal expression of the disease. Only 10 cases were detected antenatally and 20/30 cases were retrospectively analysed based on antenatal ultrasounds. Intra uterine growth retardation (IUGR) was the most frequent detectable feature (20/30). IUGR was either isolated (9/20) or associated with at least one other anomaly (11/20) including nuchal oedema, renal, cardiac, cerebral malformation, genital anomalies and polydactyly. Among them, 3/11 presented with multiple malformations (> 3 anomalies). In 5/30 cases isolated nuchal oedema (3/30), isolated cardiac (1/30), or renal malformation (1/30) was the only detected anomaly. Finally, ultrasound survey was considered normal in 5/30 cases. We conclude that SLO is frequently expressed antenatally, and we suggest giving consideration to a more systematic sterol screening when dealing with IUGR, especially when associated anomalies are detected.

RARE AND UNEXPECTED MUTATIONS AMONG IRANIAN BETA THALASSEMIA PATIENT AND PRENATAL SAMPLES DISCOVERED BY REVERSE-HYBRIDIZATION AND DNA SEQUENCING. E.

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-Thalassemia is one of the most common hereditary disorders in Iran with an extensive spectrum of mutations. While peripheral diagnostic laboratories are covering the most common mutations known to occur within their respective area, samples remaining unknown mutation are referred to our national reference laboratory for further analysis. We have analyzed 70 previously unresolved cases (thalassemia patient and prenatal samples) by a two-step approach combining reverse-hybridization and DNA sequencing of the entire β -globin gene and 5'-untranslated region. Results: In 60 (86%) of these samples a total of 23 different rare and unexpected β -globin alleles were identified. One of them was a previously unknown transition from AAG (Lys) to TAG (Stop) in codon 95. These results further emphasize the heterogeneity of β -thalassemia in Iran. Refining the knowledge about the distribution of common and rare mutations will further improve our abilities in carrier detection and prenatal diagnosis to prevent this prevalent disorder.

Genetic susceptibility to thrombophilia in women with a past history of pregnancy-induced hypertension. *J. Girouard, N. Bernard, P. Savard, J.M. Moutquin, J. Massé, J.C. Forest, Y. Giguère.* CHUQ, CRSFA, Unité de périnatalogie, Québec, Canada.

Pregnancy-induced hypertension (PIH) is a pregnancy disease characterised by hypertension occurring after 20 weeks of gestation. Some retrospective studies have suggested that women with a past history of PIH could be at increased risk of cardiovascular disease. So far, in a nested case-control study, we are evaluating the cardiovascular risk factor profile (phenotype and genotype) of women with a past history of PIH recruited prospectively 6 to 12 years ago. It is possible that susceptibility to thrombophilia could predispose women to both PIH and long-term cardiovascular disease, but this is not established yet. Polymorphisms of the Factor V Leiden (G1691A), thermolabile MTHFR (C677T), prothrombin (G20210A) and PAI-1 (4G/5G) genes were studied. Cases (n=159) were matched for age and year of delivery with controls (normal pregnancy, n = 106). All genotypes were performed by ASO-PCR. Genotypes distribution followed Hardy-Weinberg equilibrium for all polymorphisms except for the MTHFR polymorphism in cases. This disequilibrium suggests an enrichment of TT-genotype among cases but the odd ratio (OR) for this genotype compare to controls did not reach significance (OR = 1.28; C.I. 95% = 0.65 - 2.53; p = 0.59). Also, for this polymorphism, subgroups analyses based on diagnostic severity did not show any difference between groups. Carrier frequencies of the Factor V Leiden (3.2% vs 4.8 %; p = 0.75) and the G20210A prothrombin (2.5% vs 2.9%; p = 0.83) gene polymorphisms were similar between cases and controls. For the PAI-1 gene polymorphism, genotype and allele frequencies were similar between cases and controls. Using a prospective design, we did not observe any association of the commonly studied polymorphisms associated with genetic susceptibility to thrombophilia in women with a past history of PIH from the French Canadian population.

Linking pregnancy-induced hypertension to cardiovascular disease risk: polymorphisms of the lipoprotein lipase and hepatic lipase genes in French Canadian women. *N. Bernard, J. Girouard, P. Savard, J. Massé, J.M. Moutquin, J.C. Forest, Y. Giguère.* CHUQ, CRSFA, Unité de recherche en périnatalogie, Québec, Canada.

Pregnancy-induced hypertension (PIH) complicates 3-6% of pregnancies and causes increased morbidity for the mother and her fetus. Also, it is suggested that women who suffered from PIH are at increased risk of cardiovascular disease (CVD), but there is little data based on prospective designs. From a cohort of 5543 primiparous women recruited between 1990 and 1996 and diagnosed prospectively, we are studying the link between PIH and long-term risk of CVD. In an ongoing follow up nested case-control study, we are evaluating clinical and anthropometrical profiles of subjects who were pregnant 6-12 years ago, in addition to polymorphisms of the lipoprotein lipase (LPL) and hepatic lipase (HL) genes. We recruited 159 cases (PIH: 34.5 year old) and 106 controls (normal pregnancy: 35.4 year old). We found unfavorable anthropometrical (BMI: 27.5 vs 24.7 kg/m², $p = 0.0002$; abdominal circumference: 101.2 vs 94.9 cm $p = 0.0002$) and lipid (HDL-cholesterol: 1.29 vs 1.40 mmol/L, $p = 0.004$; atherogenic index: 3.8 vs 3.4, $p = 0.004$) profiles in women with a past history of PIH compared with controls. Since the frequency of LPL mutations is increased in French Canadians, we studied by ASO-PCR the risk of PIH associated with the presence of the N291S, D9N, P207L, G188E, D250N and S447X polymorphisms of the LPL gene, as well as the C-514T polymorphism of the HL gene. The combined carrier frequencies of the N291S, D9N, P207L, G188E and D250N mutations (8,1% vs 9.8%; $p = 0.79$) as well as the S447X polymorphism (11% vs 14%; $p = 0.25$) were similar between cases and controls. However, there was a trend for a decreased proportion of 514T allele carriers (HL gene) in the PIH group (odd ratio (OR) = 0.7; 0.4-1.1), especially in the preeclampsia (PIH and proteinuria) subgroup (OR = 0.5; 0.3-1.0) compared with controls. Our results suggest that the lower frequency of HL 514T carriers may explain in parts the increased risk of PIH and the unfavorable CVD risk profile in French Canadian women.

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Preimplantation Genetic Diagnosis for Cancer Predisposition. *S. Rechitsky, O. Verlinsky, A. Chistokhina, T. Sharapova, S. Ozen, C. Masciangelo, A. Kuliev, Y. Verlinsky.* Reproductive Genetics Inst, Chicago, IL.

Preimplantation genetic diagnosis (PGD) has recently been offered for couples with inherited predisposition for late onset disorders. We offered this approach for the couples at risk for producing children with cancer predisposition. Using a standard IVF procedure, oocytes or embryos were tested for different mutations predisposing to cancer, preselecting and transferring only mutation-free embryos back to the patients. The procedure was performed for patients with predisposition to familial adenomatous polyposis (maternal 13 bp and 5 bp - deletions in exon 15 and paternal nt2110 insertion G in APC gene), Von Hippel Lindau syndrome (paternal A to T substitution in nt482 of exon 1 in VHL gene), retinoblastoma, Li- Fraumeni syndrome (paternal G524A and maternal 902insC mutations in p53 tumor suppressor gene), neurofibromatosis type I (maternal Trp->Ter (TGG->TGA) mutation in exon 29 of NF1 gene) and type II (L141P mutation in exon 4 of NF2 gene), and familial posterior fossa brain tumor (maternal mutation due to G to A substitution in a donor splice site of Nt443 of exon 7 in hSNF5 gene). Overall, 20 PGD cycles were performed for 10 couples, resulting in preselection and transfer of 40 mutation- free embryos, which yielded 5 unaffected clinical pregnancies and 4 healthy children born by the present time. Despite the controversy of PGD use for late onset disorders, the data demonstrate the usefulness of this approach as the only acceptable option for at risk couples to avoid the birth of children with inherited predisposition to cancer and have a healthy child.

Intact fetal cell isolation from maternal blood using neither MACS nor flow cytometry: Improved isolation using a simple whole blood progenitor cell enrichment approach (RosetteSep™). *F.Z. Bischoff¹, D. Marquez-Do¹, D.I. Martinez¹, C. Horne², D. Dang¹, A. Burke¹, D.E. Lewis², J.L. Simpson^{1,3}.* 1) Depts OB/GYN; 2) Immunology; 3) Molecular/Human Genetics, Baylor Col Medicine, Houston, TX.

Isolation and analysis of intact fetal cells in maternal blood is an attractive method of noninvasive prenatal diagnosis. The frequently observed poor sensitivity and inconsistent recovery is compounded by few circulating fetal cells as well as loss of these fetal cells during enrichment. Optimizing selection criteria and utilizing less complicated methods for target cell enrichment is essential. Hence, MACS and other methods that minimize fetal cell loss have replaced flow cytometry methods. We report salutary results using a simple density-based depletion method that requires neither MACS nor flow separation. **OBJECTIVE:** To determine the sensitivity of fetal cell detection using a density-based method for enrichment of progenitor cells. **METHOD:** Maternal blood samples were obtained prior to amniocentesis/ CVS and then processed randomly within 24 hrs using one of two density-based enrichment methods: 1) For progenitor cell enrichment, samples (n=36; 15.3 mean gestational wks) were labeled with a RosetteSep progenitor antibody cocktail (StemCell, Tech) to remove unwanted mature T-cells, B-cells, granulocytes, natural killer cells, neutrophils and myelomonocytic cells. 2) For enrichment of CD45- cells, samples (n=16; 15.1 mean gestational wks) were labeled with RosetteSep CD45 antibody for removal of maternal white cells. Following antibody labeling, cells were separated on a single Ficoll density (1.077 gm/ml) gradient. The desired non-labeled and less dense cellular fraction was collected and analyzed by FISH using X and Y chromosome-specific probes. **RESULTS:** The detection rate with the progenitor cell enrichment approach was 55% (6/11 male cases) with 4% false-positive (1/25 female cases). No fetal cells (0/7 male cases) were detected with CD45- enrichment. **CONCLUSIONS:** Sensitivity observed using the progenitor cocktail is comparable to that obtained by a single serum analyte with low false-positive detection, and equal or superior to that found with far more laborious flow cytometry or MACS.

Phenotype-karyotype discrepancy in prenatal diagnosis of Turner syndrome: FISH detection of unusual Y chromosome rearrangements. *C. Guze*^{1,3}, *B.J. White*², *J. Kelly*⁴, *A. Anderson*³, *L. Mak*², *R. Hassan*¹. 1) Dept OB/GYN, King Drew Medical Ctr, Los Angeles, CA; 2) Quest Diagnostics, San Juan Capistrano, CA; 3) Calif State Univ Dominguez Hills, Carson, CA; 4) Quest Diagnostics at LA Metro, Van Nuys, CA.

A 27 y.o. Hispanic G5P2A2 requested an amniocentesis at 16 3/7 weeks because of a previous child with Prader-Willi syndrome due to a de novo deletion. She was aware of the low recurrence risk but because of possible gonadal mosaicism she wanted prenatal testing. Following an initial cytogenetic report of 45,X, a fetal ultrasound indicated a male phenotype. When a review of banded slides did not reveal a Y chromosome, FISH of 2 slides was done using X and Y centromeric probes (Vysis, Inc.). The FISH results showed 3 cell lines: one with a single X chromosome signal consistent with the 45,X cell line; a second with an X and a small Y with an absent long arm; and a third with an X and a dicentric Y consisting of two long arms. To assess proportions of the 3 cell lines and Y structure, FISH was performed using Y probes for the sex determining region (SRY) and distal long arm (Yq12). Both the deleted and dicentric Y were SRY positive; both q arms of the dicentric were positive for Yq12. These results from 40 interphases and 2 metaphases indicated a presumptive karyotype of 46,X,dic(Y;Y)(p11.1 or p12;p11.3)[18]/45,X[13]/46,X,del(Y)(q11.1 or q11.21)[9]. The presence of SRY positive cell lines is consistent with the observation of a male phenotype in a fetus with 45,X mosaicism. Examination by a pediatric endocrinologist following delivery revealed normal male external genitalia: rugated scrotum, descended testes and normal penis. These findings generally correlate with future normal male development with little or no risk of gonadoblastoma. The peripheral blood karyotype confirmed the presence of the 3 cell lines: 45,X[8]/46,X,del(Y)(q11.21)[7]/46,X,psu dic(Y)(p11.3)[5]. These Y rearrangements appear to be de novo as the father's chromosomes were normal. This case argues strongly for use of FISH SRY probes on any amniocentesis with a 45,X, to detect mosaicism which may confer potential for normal male development.

A randomized controlled trial (RCT) to evaluate the use of misoprostol for second trimester pregnancy termination associated with fetal malformation. *H. Akoury¹, M. Hannah¹, R. Windrim¹, D. Myles Reid^{1,2}, A. Pai^{1,2}, M. Thomas^{1,2}, G. Seaward¹, E. Winsor², G. Ryan¹, L.E. Ferris¹, T. Einarson¹, A. Willan¹, M. Thomas³, D. Chitayat^{1,2}.*
1) Obstetrics and Gynecology, Mount Sinai Hospital, Toronto, Ontario, Canada; 2) The Prenatal and Medical Genetics Program, Mount Sinai Hospital, Toronto, Ontario, Canada; 3) York University,McLaughlin College, Toronto, Ontario, Canada.

Objective: To compare the effectiveness and safety of misoprostol administered orally (OM) or vaginally (VM) to intra-amniotic injection of prostaglandin F₂ (IAPG) **Methods:** A multi-centre RCT of 3 groups with 217 patients. The primary outcome was the time from start of treatment to placental delivery and the secondary outcomes were the incidence of major and minor maternal complications. OM and VM groups received misoprostol 400 microgram every 4 hr. for 24 hr. IAPG group received 40 mg PGF₂alpha intra-amniotically followed 4 hr. later by oxytocin infusion at 166 mU/min for 24 hr. or until delivery. We performed one interim analysis after 1/3 of patients were enrolled. Before the start of the trial, we designed criteria to stop part or all the trial. **Results:** There were no statistical differences among the three groups in baseline characteristics or in the incidence of major and or minor maternal complications. The interim analysis showed OM had statistically significant longer time to placental delivery compared with VM and IAPG. Therefore, we stopped recruitment in OM and completed recruitment in the other two groups in March 2001. At study completion, mean and standard deviation of the primary outcome in hours was 18.3±8.2 for VM, 21±10.2 for IAPG and 30.5±14.4 for OM. OM showed statistically significant difference in time to placental delivery compared with VM, $p < 0.001$ and IAPG, $p < 0.001$ with no statistically significant difference was found between VM and IAPG, $p < 0.33$. **Conclusion:** Results of this RCT suggest that VM is an acceptable method for second trimester pregnancy termination. However, oral misoprostol, as used in this study, is not an effective method. More research in oral misoprostol use may be warranted.

A simple and sensitive scoring system to identify fetal erythroblasts in maternal blood. *D.H. Cha, B. Hogan, R.M. Bohmer, D.W. Bianchi, K.L. Johnson.* Division of Genetics, Departments of Pediatrics and Obstetrics and Gynecology, Tufts-New England Medical Center, Boston, MA.

Background: Nucleated red blood cells (NRBCs) of fetal origin may have distinguishable characteristics from that of maternal NRBCs in both nuclear morphology and hemoglobin staining properties. However, these differences have yet to be quantified into a definable assay. Here, we present a scoring system using four parameters considered separately (nuclear roundness, nuclear morphology, gamma hemoglobin staining intensity, and peripheral brightness of the stained cytoplasm) to address this issue. **Methods:** NRBCs were isolated from four maternal blood samples by a density gradient, CD15/45 depletion, and a flow cytometry based anti-gamma hemoglobin positive selection after elective termination of a trisomy 21 male fetus (47,XY,+21). All cells were deposited onto microscope slides, and every NRBC was analyzed according to the scoring system. Each of the four individual parameters was given a value from 0 to 3 points and a combined score was obtained for each cell (range 0-12). FISH using X and Y specific probes was performed to determine whether the cell was maternal or fetal.

Results:

Combined score	5 or less	6	7	8	9	10	11 and 12
% fetal cells	5.7	19.2	37.5	63.6	71.4	91.7	100

Conclusions: Fetal NRBCs have a characteristic morphology and gamma hemoglobin staining appearance that makes them distinguishable from maternal NRBCs. The scoring system presented here is a sensitive and simple method to distinguish fetal NRBCs from adult cells in maternal blood and may have applications for both basic research into differences between fetal and adult cells as well as future clinical utility for noninvasive prenatal diagnosis. In addition, these defined parameters may also provide computational classifiers for the automated detection of fetal cells from maternal blood.

Fetal cells from maternal blood: High density gradient *versus* magnetic separation from whole blood. *R.M. Bohmer, H. Stroh, D.W. Bianchi.* Pediatrics, Tufts New England Medical Cent, Boston, MA.

Isolation of fetal cells from maternal blood using density gradient separation causes substantial loss of fetal nucleated red cells (NRBC), because of the similar densities of mature NRBC and erythrocytes. Using spikes of fetal into adult blood, we quantitated the recovery of HbF+ fetal NRBCs using flow sorting (FACS) and subsequent FISH to identify fetal cells by gender. We increased the Percoll gradient density to the maximum that would keep contaminating erythrocyte numbers small enough to allow subsequent flow sorting. At this density, up to 10 times more fetal nucleated red cells were recovered than with the standard Ficoll 1.077 or 1.083. Unfortunately, this optimal density varied with blood donor and blood storage history, and recovery was still only 40-60% of spiked fetal nucleated red cells. Therefore, we tested if we could avoid the density gradient altogether and isolate CD71+ cells from whole blood using magnetic beads. This was also followed by HbF+ fetal cell quantitation via FACS and FISH. Different commercially available magnetic bead systems were compared. The best fetal nucleated red cell recovery via whole-blood procedure was approximately equal to the recovery by maximum Percoll density. Further fine-tuning of both methods, as well as trials on maternal blood samples, may help decide which is the best approach to maximize fetal nucleated red cell isolation from maternal blood.

Prenatal diagnosis of mosaicism for structurally abnormal Y chromosomes: need for thorough cytogenetic and clinical evaluation. *L. Cartier, J-P. Capolicchio, A. Duncan, V. Desilets.* Prenatal Diagnosis Program, McGill Univ Health Ctr, Montreal, PQ, Canada.

Predicting the phenotypic consequences of different structural aberrations of chromosome Y is usually complicated by varying degrees of mosaicism (45,X cell lines). Few prenatally diagnosed cases have been reported.

We report on the outcomes of two cases of mosaicism for structurally abnormal Y chromosomes: mos 46,X,r(Y)(p11.3q12)[18]/45,X[5]/46,XY[5].ish r(Y)(p11.3q12)(SRY+) and mos 45,X[11]/46,X,idic(Y)(q11.2)[10].ish idic(Y)(wcpY+,DYZ3+,SRYx2) detected prenatally after amniocentesis done for advanced maternal age. High level resolution ultrasound at mid-trimester revealed well differentiated male external genitalia without anatomic features of Turner syndrome. Fetal echocardiogram was normal. Ultrasound at 32 weeks revealed testes in the scrotum without hypospadias. On postnatal examination, both babies had normal external genitalia and were not dysmorphic. Cord blood karyotype revealed the persistence of the 45,X cell line.

A wide spectrum of clinical manifestations including female phenotype with short stature and other characteristics of Turner syndrome, male phenotype with azoospermia, and ambiguous genitalia have been described in postnatally diagnosed patients. In studies of prenatally diagnosed 45,X/46XY mosaicism over 90% of cases have a grossly normal male phenotype, the remaining cases having ambiguous genitalia or female genitalia with Turner syndrome. There is a risk of about 15% for gonadoblastoma. This suggests that many patients with structural aberrations of chromosome Y go undetected. The 45,X cell line, regardless of the presence of Yp or a region including SRY carries an increased chance for that individual to be a phenotypic female with Turner features or to have ambiguous genitalia. The present cases illustrate the role of ultrasound in predicting the impact of a 45,X cell line and the role of genetic counselling in ensuring appropriate prenatal follow-up and postnatal management.

Simplifying Fetal Cell Analysis in Maternal Circulation. *P. Bayrak Toydemir*¹, *M.B. Fiddler*², *E. Pergament*^{1,3}. 1) Northwestern Reprogenetics Inc, Chicago, IL; 2) DePaul University, Chicago, IL; 3) Northwestern University School of Medicine, Chicago, IL.

Thirty samples of maternal blood, between 10 and 22 weeks gestation, were studied to determine the effectiveness of enriching fetal cells by a rapid and simple centrifugation procedure. **Material and Methods:** Five ml of heparinized blood was centrifuged at 1960 g-min to obtain a plasma fraction containing a low density cell population. Except in one case, all samples of blood were obtained prior to any invasive prenatal procedure (i.e., CVS or amniocentesis). Cells in this fraction were then re-centrifuged 2500 rpm and washed twice in PBS. The presence of fetal cells was determined by FISH analysis for Y-chromosome and/or chromosome abnormalities of the chromosome 18, 21, X, and/or Y. Blood samples were immediately processed and fixed in test tubes; samples from women carrying a cytogenetically abnormal fetus, as determined by 1-day FISH analysis, were given priority for analysis. Controls consisted of blood samples taken from non-pregnant women (2) and women carrying a female fetus (7). **Results:** Fetal cells, determined by the presence of a Y chromosome but no abnormalities, were detected in 77% of maternal samples (10 of 13 cases). When a chromosome abnormality was present, fetal cells were detected in 7 of 8 cases (87.5%). In one instance, a mosaic (47,XY,+20/46,XY) was not detected. In abnormal cases fetal cell frequency was 1 cell in 522 maternal cells; in normal male cases the frequency was 1 in 1838 maternal cells. Negative control cases did not have any Y-fluorescent cells. **Discussion:** These results demonstrate the potential for obtaining an enriched sample of fetal cells from maternal blood by a simple centrifugation; further studies to refine the conditions for centrifugation with the intent of improving yield are underway. In addition, these results affirm previous observations that cytogenetically abnormal fetal cells are found in maternal plasma with greater frequency than apparently normal fetal cells and that this frequency, with further improvement of enrichment techniques, may approach 100%.

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Molecular and Prenatal Diagnosis of Spinal Muscular Atrophy in families from North India. *A. Kesari, M. Mukherjee, S.R. Phadke, B. Mittal.* Medical Genetics, Sanjay Gandhi Postgraduate Ins, lucknow, UP, India.

Spinal muscular Atrophy (SMAs) forms a clinically and genetically heterogeneous group of autosomal recessive neuromuscular disorders leading to symmetrical muscle weakness and atrophy. The SMN gene responsible to cause SMA is present in two homologous copies, SMNt (telomeric) and SMNc (centromeric). In this report, we present the molecular analysis of exons 7 and 8 of SMN gene in patients (with suspected clinical diagnosis of SMA) from 42 North Indian families by using PCR-RFLP. Out of these, 18 have deletion of both exons 7 and 8 of SMNt, while two patients showed a deletion of only exon 8. Unlike in different populations where 90-95% SMA cases had SMNt deletion, our population showed SMNt deletion only in 43% patients. In the course of our study we also came across an unusual case, showing a homozygous deletion of SMNc gene. In addition to molecular analysis, prenatal diagnosis was performed in four families with previous history of SMA. One foetus was affected and the other was normal in the two families where mutation had been found in the surviving sibs. The families were counselled accordingly. The low frequency (43%) of the SMNt gene deletion in our population can be attributed to the presence of point mutations that might be more prevalent in North Indian patients. The results also add to the genetic heterogeneity suggesting that the patients may be SMA variants with gene deletion out side 5q13.

Normal Amniocentesis Hiding an Age Related Abnormality: Consider Adding UPD 15 Testing for Prader-Willi Syndrome to Prenatal Diagnosis for Advanced Maternal Age. *L. Medne, D. Stewart, E.H. Zackai.* The Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA.

We diagnosed two infants in the newborn period with Prader-Willi syndrome (PWS) caused by maternal UPD 15. Both mothers had undergone an amniocentesis because of advanced maternal age (AMA) and in both cases karyotypes were normal. Case 1 was born to a 39 y/o G3 P1@2 (TAB for prior trisomy 21) mother by C-section due to a breech presentation. The infant had significant hypotonia and feeding difficulties requiring NG feeds. Case 2 was born to a 43 y/o G4 P0@1 (1 TAB, 2 SAB) mother by a C-section due to a breech presentation. The infant had IUGR, cryptorchidism, hypotonia and feeding difficulties. Maternal UPD 15 causes 25% cases of PWS cases, and the rate of maternal UPD 15 increases with age (Mascari et al, 1992). Under 35 years of age, the incidence of maternal UPD is 140 times less than that of trisomy 21, but the margin narrows with advancing maternal age. At age 40, the incidence of maternal UPD 14 is 1/3400 live births, only 68 times less than the incidence of trisomy 21 (Robinson et al., 1996). The mothers in our two families, who underwent amniocentesis to rule out maternal age related chromosomal abnormalities, would have terminated an affected pregnancy. They feel that their children should have been diagnosed prenatally because of the known associated age related factor in maternal UPD 15. We propose that routine screening for maternal UPD 15 in CVS and amniocentesis done for AMA be considered.

Analysis of Fetal Cells in Maternal Blood: Influence of Apoptotic Status and FISH Probes. *S. Mergenthaler, T. Babochkina, G. De Napoli, S. Hristoskova, W. Holzgreve, S. Hahn.* Obstetrics and Gynecology, University of Basel, Basel, Switzerland.

The use of fetal erythroblasts enriched from the maternal circulation provides a tantalising risk free alternative for prenatal diagnosis. The scarcity of these fetal cells, which even after enrichment may only be of the order of 0.001%, implies the tools used for their analysis have to be optimal; since if this method has small false positive rate, of the order of fetal erythroblast frequency, a significant number of the fetuses would be scored incorrectly. In our previous studies performed in the NIH funded NIFTY study, where we have mainly used FISH for the X and Y chromosomes as a model system, we have noted that the use of indirectly labelled FISH probes resulted in high false positive rates (over 23%). By switching to a commercial source of directly labelled FISH probes, we recently observed that the false positive rate could be lowered to less than 8% with a concomitant increase in sensitivity to over 72%. A further important aspect is the recent observation that almost half of these erythroblasts have an apoptotic phenotype, by having fragmented DNA in their nuclei (TUNEL positive). This raised the concern of whether these nuclei will be suitable for analysis by FISH, or whether they may be more suitable for analysis by single cell PCR. We have now determined that the efficiency of FISH analysis is not influenced by the apoptotic phenotype of the erythroblast interrogated. We did, however, note that the Y chromosome FISH probe was significantly less efficient in hybridising effectively to erythroblast nuclei in comparison with the X chromosome probe, in that on average only 73% of all male erythroblasts were identified correctly in contrast to almost 94% of all female erythroblasts. Our result, therefore, suggests that the current apparent lack of sensitivity for the identification of male fetal erythroblasts enriched from the maternal circulation may in part be attributable to the incorrect choice of a suitable FISH probe for the Y chromosome. We are, therefore, investigating which Y specific loci are most suitable.

Non detection of the fetal SRY sequence in healthy women who have had male offspring. *C. Le Marechal*^{1,2,3}, *C. Benech*¹, *C. Ferec*^{1, 2, 3}. 1) EFS Bretagne, Brest, France; 2) INSERM EMI 0115, Brest, France; 3) Universite de Bretagne Occidentale, Brest, France.

The discovery of fetal cells and free fetal DNA in the maternal bloodstream opened the field for performing non-invasive prenatal diagnosis (NIPD) using maternal venous blood. Risks for the fetus linked to invasive procedures (amniocentesis or CVS) could be eliminated, and with the recent advances in molecular biology techniques, the study of fetal DNA could be performed earlier in pregnancy. Previous studies have shown that fetal cells in the maternal circulation persist even decades after delivery (Bianchi 1996), whereas free fetal DNA disappears rapidly after birth (Lo 1999, Costa 2001). Until now, the rapid clearance of free fetal DNA from maternal serum and plasma allowed one to think that only a pregnancy in progress could be studied. Recent contradictory findings (Invernizzi 2002) have shown that fetal DNA (SRY sequence) is detectable in 20% of healthy women's serum up to 40 years after birth of their last male child. These findings modify the feasibility of an NIPD from maternal blood because of the possibility of false positive results linked to former pregnancies. In order to evaluate this approach, we extracted DNA from 1ml of serum (QIAamp ultraSens™ Virus kit- QIAGEN) from 33 healthy women with male offspring. The amount of time since the birth of their last male child ranged from 1.5 to 37 years (mean 13.9 years). TaqMan real time quantitative fluorescent PCR was performed to detect the SRY sequence. The final PCR volume was 50µl with 15µl of the extracted DNA. Pregnant women carrying male fetuses were chosen as positive controls and young healthy women who had never been pregnant were chosen as negative controls. Under these conditions we found no SRY sequence in the studied group. Thus, according to our conditions, we conclude that previous pregnancies do not interfere with serum free fetal DNA analysis performed for NIPD.

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Analysis of Cystic Fibrosis and Ashkenazi Jewish Disease Mutations by Multiplexed Elongation of Allele-Specific Oligonucleotides Displayed on Custom Bead Arrays. *G. Hashmi¹, Y. Song¹, M. Seul¹, L. Edelmann², R. Kornreich², R.J. Desnick².* 1) BioArray Solutions Ltd, Piscataway, NJ; 2) Mount Sinai School of Medicine, New York, NY.

A novel BeadChip assay system for the high throughput multiplexed analysis of mutations and polymorphisms has been evaluated for an augmented ACMG panel comprising the most common ~40 Cystic Fibrosis mutations. In addition, an Ashkenazi Jewish disease panel also has been developed using this system to detect common mutations known to cause Tay-Sachs, Canavan, Gaucher, Niemann Pick, Bloom Syndrome, Fanconi Anemia, Familial Dysautonomia and Mucopolysaccharidosis IV. In elongation-mediated multiplexed analysis of polymorphisms (eMAP), developed at BioArray Solutions, allele-specific oligonucleotides (ASO) containing variable 3'-terminal sequences are attached to color-coded beads which are in turn arrayed on silicon chips. Elongation products for mutated and normal sequences are simultaneously detected by instant imaging of fluorescence signals from the entire array using BioArray Solutions Array Imaging System (AIS). The design flexibility and convenience of implementing BeadChip assay system facilitates simultaneous screening for multiple alleles. Our results of a comparative study with an ASO dot blot method using patient samples demonstrates the sensitivity, specificity and reproducibility of the BeadChip assay system.

Factors affecting performance of prenatal genetic testing by Israeli Jewish women. *C. Sher*¹, *O. Romano*², *T. Shohat*². 1) Institute of Medical Genetics, Asaf Harofeh Medical Center, Israel; 2) ICDC, Ministry of Health, Israel.

The number of prenatal genetic tests that are being offered to women is constantly increasing. In Israel some of the prenatal tests are state subsidized (eg amniocentesis for women older than 35 years) or privately paid for (eg molecular genetic carrier testing). However, there is little national data as to who is performing the tests and the reasons for doing or not doing so. In a one day survey conducted in all the maternity departments in Israel we evaluated the proportion of Jewish women in Israel who perform the various prenatal genetic tests and the factors affecting the performance of these tests. Of the 377 women interviewed, 60.9% performed the Triple Test (TT), 50.8% women older than 35 years performed amniocentesis, while 63.3% and 24.3% performed Tay-Sachs (TS) and Fragile-X (FX) carrier testing respectively. In a stepwise regression analysis being secular was a significant factor associated with performing TT, amniocentesis, TS and FX testing. Higher income was significantly associated with performing all these tests. Having < 4 children was associated with performing amniocentesis, TS and FX testing. Women with a supplementary medical insurance performed significantly more FX tests. The main reason for the women not performing amniocentesis or the TT was for religious or moral grounds (53.3% and 67% respectively). The main reason for not performing TS or FX testing was that they were not referred for the tests (76% and 82% respectively). In view of the low performance rate of the recommended tests, consideration should be given to providing first trimester prenatal diagnosis to the religious groups, state subsidized FX testing and educating the primary care givers about the importance of prenatal genetic testing. The information from the present study is vital for the planning of equitable prenatal genetic services and provides guidelines for the implementation of such services in other countries.

Fetal DNA Diagnosis from Maternal Blood - PEP-TaqMan PCR Analysis of A Single Nucleated Erythrocyte (NRBC) -. *H. Takabayashi¹, M. Kita¹, K. Ikawa², k. Yamafuji³.* 1) Medical Research Institute, Kanazawa Medical University, Kahoku, Ishikawa, Japan; 2) Ishikawa Health Service Association, Kanazawa, Ishikawa, Japan; 3) Ukita Hospital, Kanazawa, Ishikawa, Japan.

The development of a real-time quantitative TaqMan PCR assay permits the high quality analysis of DNA or RNA. With the TaqMan PCR, Lo. et al. were successful in measuring the concentration of fetal DNA in maternal plasma and serum. Using ABI PRISM 7700 System (PE Applied Biosystems), we tried to detect the target DNA sequence of SRY and beta actin locus in the PEP (primer extension preamplification) products from a single cell. Single NRBCs (nucleated red blood cells) were retrieved from cord blood smear of male fetus using a micromanipulator. The detection rates of beta actin and SRY locus were 55% and 35%, respectively. Quantitative results of PEP products from single NRBCs were 0.5-59.5 (m=4.8) copies in beta actin and 2.4-278.4 (m=17.8) copies in SRY locus. Our quantitative data show the concentration of a single locus DNA amplified by PEP from a single NRBC. The advantages of PEP-TaqMan PCR system are high specificity and sensitivity. This system seems a powerful tool for fetal DNA diagnosis from maternal blood.

More cell-free fetal DNA from maternal plasma is recovered using vacuum pump preparation: A step toward more accurate fetal DNA analysis. *J.L. Simpson^{1,3}, D. Dang¹, D. Marquez-Do¹, C. Horne², A. Burke¹, D.E. Lewis², F.Z. Bischoff¹.* 1) Depts OB/GYN; 2) Immunology; 3) Molecular/Human Genetics, Baylor Col Medicine, Houston, TX.

Prenatal diagnosis of fetal chromosomal aneuploidies and single gene mutations is now feasible by analysis of cell-free DNA extracted from maternal blood. In particular, clinical diagnostic applications have been proposed using real-time PCR for quantitative analysis of cell-free fetal DNA in maternal plasma. Although cell-free fetal DNA is relatively abundant in maternal plasma, its admixture with maternal cell-free DNA can interfere with fetal DNA detection. Standardized protocols to combat this problem have not been developed, and methods for routine plasma DNA extraction have not been established. **OBJECTIVE:** To determine whether a vacuum manifold results in greater extraction of total and fetal DNA, as detected by real-time PCR. **METHODS:** Peripheral blood was obtained from women (n=28) prior to CVS or amniocentesis at 10.1 to 23.1 weeks gestation (mean 14.8 wks). Blood collected in vacutainers containing ACD was processed within 16 hrs. DNA extraction and purification was performed using the QIAamp DNA blood mini kit (Qiagen) with (n=19) or without (n=19) the vacuum manifold. Real-time PCR was performed using the TaqMan assay to detect the FCY (DYS1) and GAPDH (control) locus. Fetal DNA concentration (Geq/ml plasma) was determined using the Sequence Detection System software (PE Applied Biosystems). **RESULTS:** Mean concentration of total (GAPDH) and fetal (FCY) was significantly higher (P=0.03) among vacuum-processed samples (1,268 Geq/ml and 30.9 Geq/ml) than in samples processed without vacuum (417 Geq/ml and 17.6 Geq/ml). With vacuum, sensitivity for identifying fetal gender was 90% with 100% specificity in correctly identifying fetal gender; with no vacuum, sensitivity and specificity were 70% and 88%. **CONCLUSION:** Utilization of the vacuum manifold not only optimized recovery of total cell-free fetal DNA, but also resulted in greater sensitivity of detecting fetal DNA sequences. Vacuum manifold should be incorporated into protocols for optimal recovering cell-free DNA from maternal plasma.

Maternal serum cell-free fetal DNA levels are increased in cases of trisomy 13 but not trisomy 18. T.

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Cell-free fetal DNA in the maternal circulation is a potential noninvasive marker for fetal aneuploidies. In previous studies using Y DNA as a fetal-specific marker, circulating levels of fetal DNA were elevated in women carrying trisomy 21 fetuses. The goal of this study was to determine whether cell-free fetal DNA levels in the serum of pregnant women carrying male fetuses with trisomies 13 or 18 are also elevated. Serum from five cases of T13 and five cases of T18 were studied. Each case was matched for fetal gender, gestational age and duration of freezer storage to 5 control pregnancy samples presumed to be euploid after medical record review. Real-time quantitative PCR amplification of DYS1 was performed to measure the concentration of male fetal DNA present. Unadjusted median serum fetal DNA concentrations were 97.5 genome equivalents per milliliter [GE/mL] (29.2-187) for the trisomy 13 cases, 31.5 GE/mL (18.6-77.6) for the trisomy 18 cases and 40.3 GE/mL (3.7-127.4) for controls. Fetal DNA levels in trisomy 13 cases were significantly elevated ($p=0.016$), by analysis of variance of the ranks of values within each matched set. In contrast, fetal DNA levels in trisomy 18 cases were not different from controls. Second trimester maternal serum analytes currently used in screening do not identify fetuses at high risk for trisomy 13. Fetal DNA may facilitate noninvasive screening for trisomy 13 provided a gender-independent fetal DNA marker can be developed.

Subtelomeric rearrangement: low rate of spontaneous abortion in balanced carriers. *M.K. Tonnesen, L. Medne, K.L. Russell, J. Ganesh, I.D. Krantz, N.B. Spinner, E.H. Zackai.* Division of Human Genetics and Molecular Biology, The Children's Hospital of Philadelphia and University of Pennsylvania School of Medicine, Philadelphia, PA.

The rate of spontaneous abortions (SABs) in carriers of balanced translocations was found to be 25-50% (Neri et al., 1983). This led to multiple large scale studies of couples with recurrent SABs (>2), which revealed that in 3-5% (Tharaperl et al., 1985; Campana et al., 1986) there is one partner with a balanced chromosomal translocation. In the absence of a study for subtelomeric rearrangements in couples with recurrent SABs, we looked at the SAB rate in couples where one partner was identified to be a carrier of a balanced subtelomeric translocation ascertained because of an affected child. This study includes 5 couples identified at our center [t(15;19)(p13;q13.3); t(13;19)(p12;q13.3); t(13;18)(q34;q23); t(1;9)(p36.3;q34)] and 52 couples from the literature. Of the 139 total pregnancies of carriers, there were 11 SABs for a rate of 7.9% (6.9% (5/72) in female carriers and 8.9% (6/67) in male carriers). This SAB rate is significantly below the 25-50% SAB rate observed in carriers of cytogenetically detectable translocations and below the general population 10-15% SAB rate (US Department of Health and Human Services, 1982). We postulate that the observed low SAB rate in subtelomeric translocation carriers could be attributed to the high viability of unbalanced conceptions due to the very small chromosomal imbalance (Young, 1991). The data predict that testing for subtelomeric rearrangements in couples with recurrent SABs will have a low yield.

Prenatal diagnosis of Down syndrome - ten years' experience in the Israeli population. *M. Shohat*¹, *J. Zlotogora*², *Israeli consortium for Down syndrome prevention*³. 1) Dept Medical Genetics, Rabin Medical Ctr, Petah Tikva, Israel; 2) Dept Medical Genetics, Ministry of Health, Israel; 3) Medical Genetics Institutes of other Medical Centers, Israel.

Maternal serum biochemical markers, the major parameter introduced between 1990 and 1995, was supplemented with new promising modalities - nuchal translucency, soft signs on targeted ultrasound at 14-16 weeks, and first trimester biochemical markers - between 1995 and 2000. This study evaluated the effectiveness of a Down syndrome (DS) prevention program among the Israeli Jewish population between 1990 and 2000. We collected data on the total number of prenatal tests performed on Israeli Jewish women, DS cases detected prenatally and DS live births in Israel during these years. We also studied the use of the various newer screening tests for DS in 1990, 1992 and 2000. Between 1990 and 1995, the uptake of prenatal testing for DS in the Israeli Jewish population increased from 11.3% to 21.6% - this was accompanied by an improvement in the DS detection rate from 53% to 70%. However, between 1996 and 2000, even though new DS screening methods were introduced, the rate of uptake remained similar (20.7% to 19.8%) and the DS detection rate in the Jewish population decreased to 61% in 2000. The total cost per DS case detected increased from \$47,971 in 1990 to \$75,229 in 1992, and to \$190,171 in 2000. A significant improvement in the DS detection rate between 1990 and 1995 was associated with a significant increase in the amniocentesis rate - both are attributed to the introduction of the maternal serum biochemical marker tests. Unexpectedly, the introduction of new genetic modalities for the assessment of DS risk in Israel between 1995 and 2000 did not improve the overall detection rate or reduce the relatively high amniocentesis rate, but was accompanied by an increased cost per case detected.

The First Trimester Population Screening: Do We Need More Evidence? *G.L. Tsukerman^{1,2}, I.A. Kirillova^{1,2}, N.B. Gusina², H.A. Zinkevich², G.A. Krapiva², L.M. Lishtvan², O.V. Pribushenya², N.A. Venchikova², L.A. Savenko², H.I. Golovataya²*. 1) Joint Research Program, Reproductive Genetics Institute, Chicago, IL; 2) Institute for Hereditary Diseases, Minsk, Belarus.

During Joint Research Program 70,200 unselected pregnant women were screened in the first trimester by transabdominal or transvaginal ultrasound. The majority of them were screened between 10.5 and 13 weeks of gestation. There were 6.3% of women of 35 years and older. 1,504 empty sacs and non-viable fetuses, 680 multiple pregnancies and 182 cases of fetal malformations were revealed. 126 of them were neural tube defects. Practically, no cases of anencephaly were missed. Besides that, 16,000 serum samples from unaffected pregnancies and 42 Down syndrome cases were tested for AFP, Free b-hCG and partly for PAPP-A. The median level of AFP, Free b-hCG and PAPP-A in single unaffected pregnancies for each CRL was derived. The expected detection rate for different marker combinations was determined. A modelling exercise for screened population with maternal age, NTT, AFP, PAPP-A and Free b-hCG predicts a 92% detection rate for Down syndrome in FTS at 5% FPR. 10, 259 of unselected pregnant women were tested according to the protocol during the last two years. 93% of pregnant women were screened at CRL of the fetus between 38 and 70 mm. The risk of 1:360 at the expected date of delivery was chosen as the cut-off for an invasive procedure. 16 cases of trisomy 21 were detected with the expected number 14. In the last three month 2, 520 a sagittal views of the fetal face were obtained and the absence or presence of nasal bone was evaluated. The nasal bone was absent in three cases and one of them had trisomy 21. Taking into consideration the results of the other programs (Nicolaides K. et. al, 2002; Cicero S. et.al., 2001; Spencer K. et al., 2000; Krantz D. et al., 2000) it is beyond reasonable doubt that the first trimester population screening for various fetal abnormalities is not just desirable, but it should also become the standard of care in prenatal diagnosis.

Acellular fetal DNA in maternal plasma does not stem from the transplacental passage of fetal cells. X. Zhong, W. Holzgreve, S. Mergenthaler, S. Hristoskova, S. Hahn. Obstetrics & Gynecology, University of Basel, Basel, Switzerland.

A recent major advance in the non-invasive risk free determination of fetal genetic traits has been the discovery of acellular fetal DNA in the maternal circulation, by which means it has become possible to determine fetal genetic loci such as RhD with very high levels of accuracy. Currently the origin of this acellular fetal DNA in the maternal circulation is currently unclear and it has been speculated to result either from the shedding of apoptotic material from the placenta or from the apoptosis of fetal cells which have entered the maternal circulation. This latter supposition is largely based on the observation that numerous fetal erythroblasts exhibit an apoptotic phenotype, in that their nuclei contain fragmented DNA. This hypothesis is, furthermore, supported by reports indicating that elevated numbers of fetal erythroblast and acellular fetal DNA concentrations are present in pregnancies affected by certain pathologies, such as preeclampsia. In order to address this question we have examined fetal erythroblast numbers, following enrichment and acellular DNA concentrations, by real-time PCR, in the same maternal blood samples. Our data, obtained both from the analysis of both normal and pathologically affected pregnancies, indicates that no correlation exists between these two fetal cellular and molecular species. This result was most striking in pregnancies affected by onset of pre-term contractions, where we were able to detect significant elevations in acellular fetal DNA concentrations but not any concomitant increase in the trafficking of fetal erythroblasts. Consequently, our data suggest that an alternative cell type is the source of acellular fetal DNA, most probably of placental origin. It also appears that the release of cell acellular DNA from this cell type is affected by pathological placental conditions which are not associated with an increase in fetal cell trafficking.

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Cellfree fetal DNA in the maternal circulation after amniocentesis. *O. Samura, M. Hyodo, N. Miharu, K. Ohama.*
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Our aim was to investigate whether amniocentesis increases the concentration of cell free fetal DNA in maternal serum. Twenty-four patients who had singleton male fetuses and were undergoing amniocentesis between 15 and 17 weeks of gestation were recruited. Five patients who had singleton female fetuses were recruited as controls. Maternal venous blood samples were taken before and within 10 minutes after amniocentesis. DNA was extracted from the serum samples. The amount of fetal DNA was quantified by calculating the concentration of the SRY gene sequence from the Y chromosome after amplification by polymerase chain reaction (PCR). The change in the concentration of the amplified SRY gene sequence before and after amniocentesis was compared by a paired sample t-test. There was a significant increase in the concentration of fetal DNA in maternal serum after amniocentesis (7.18±6.38copies per milliliter before amniocentesis ; 11.89±11.24 copies per milliliter after amniocentesis; p=0.011). Amniocentesis imposes a significant disturbance to the maternal- placental interface. Fetal DNA is a sensitive marker that is useful in the assessment of fetal-maternal hemorrhage.

Fetal Hepatic Calcifications: Prenatal Diagnosis and Outcome. *M.J. Simchen¹, A. Toi³, M. Bona¹, F. Alkazaleh², G. Ryan², D. Chitayat¹*. 1) The Prenatal Diagnosis and Medical Genetics Program; 2) Departments of Obstetrics and Gynecology; 3) Department of Medical Imaging.

Objective: To provide information on the etiologies and postnatal outcome of fetuses detected with liver calcifications on prenatal ultrasound. **Study design:** Cases with fetal liver calcifications seen between 1992-2001 were evaluated. A detailed fetal ultrasound for associated abnormalities, maternal TORCH analysis and parvovirus B19 serology, as well as parental CF mutation analysis were performed, and amniocentesis was offered in all cases. All cases born alive were examined and followed. **Results:** 61 pregnant women with fetal liver calcifications were identified. 40/61 cases had additional fetal abnormalities while 21/61 were isolated. 11/61 (18%) patients had abnormal karyotypes: 4-Trisomy 13; 2-Trisomy 21, 1 each Trisomy 18, 45,X 4p-22q+ and 8p+. 10/11 cases with abnormal karyotypes had other ultrasound abnormalities. 2 cases had intrauterine infection, one CMV and one parvovirus B19 infection. 18/40 patients underwent pregnancy termination, one fetus died in-utero, one newborn died postnatally and 2 suffered neurological sequelae. Of 21 cases with isolated liver calcifications, one fetus had parvovirus B19 infection and one infant had trisomy 21. Remaining infants had a good outcome. **Conclusions:** Fetal liver calcifications are relatively common. Isolated cases have a good prognosis after aneuploidy and infection have been ruled out. However, additional major abnormalities present a risk for chromosomal abnormalities, mainly trisomy 13.

Prenatal molecular diagnosis for infantile and late-infantile neuronal ceroid lipofuscinoses. *N. Zhong*^{1,2,3}, *W. Ju*^{1,2}, *D. Moroziewicz*^{1,2}, *W.T. Brown*². 1) SCL-Molecular Neurogenetic Diagnostic Laboratory; 2) Dept. Human Genetics, New York State Inst Basic Res, Staten Island, NY; 3) Dept. Neurology, SUNY-Downstate Health Science Center, Brooklyn, NY.

Infantile and late-infantile neuronal ceroid lipofuscinoses (NCL1 and NCL2) have been determined to result from genetic deficiency of gene CLN1 and CLN2, respectively. Applying molecular analyses may offer prenatal diagnosis for families affected by NCL1 or NCL2, in whose mutations have been identified. In this study, six pregnancies, three from NCL1 families and three from NCL2 families, were analyzed. In the NCL1 cases, two pregnancies were from the same family that both parents carry a mutation of 451C®T in CLN1 gene. DNA testing was done on CVS of both pregnancies and results showed that both fetuses were carrier for this mutation. In the second family, the father who had an affected NCL1 child from previous marriage was found to carry the 451C®T mutation. Prenatal diagnostic analyses of amniotic fluid cells showed the fetus does not carry this mutation, indicating a normal pregnancy. For NCL2 cases, testing R208X mutations were performed in two families. Both parents carrying R208X were found in one family. In the second family, R208X was found in the mother but the second mutation was not identified within CLN2 cDNA region from the father who had been determined to be a carrier by TPP1 enzymatic assay. Testing R208X mutation in this two cases showed both fetuses were carriers. In the third family, parents were identified to be carriers of either IVS5-1C or IVS5-1A mutation. However, the fetus carries neither mutation and is normal. All results obtained from prenatal tests were confirmed by postnatal tests. Our studies indicate that DNA testing may provide a definitive prenatal diagnosis for NCL disorders.

Increasing incidence of abdominal wall defects in the State of Illinois: trends and distribution suggests a common herbicide (2,4-dichlorophenyl-p-nitrophenylether) as a plausible candidate teratogen. *n.j. nadkarni¹, r.f. hume¹, t. egler², j. smith¹, p. prochnicki¹, h. kaufman¹, m. leonardi³, r. pearl³, p. Ittman¹, l.s. Martin¹.* 1) Medical Genetics, Neonatology and MFM, Rockford Health System, Rockford, IL; 2) Illinois Department of Public Health, Springfield, IL; 3) MFM and Pediatric Surgery, UIC.

Several centers have perceived an increased incidence of abdominal wall defects in agrarian communities in our region. An teratogen, 2,4-dichlorophenyl-p-nitrophenylether (DCNPE) is a congener of an herbicide in common use. Could the peculiar regional distribution be attributed to happenstance, or teratogen exposure? The purpose of this study is to examine the relationship between the development of congenital birth defects and a possible causal link to herbicide exposure in particular geographic regions of Illinois. We initiated a feasibility study to collect data from several disparate state sources to affirm the perceived increased incidence of congenital anomalies, review literature to ascertain credibility of biologic effect of putative agent, and compare distributions of anomalies and common use of herbicide. The Adverse Pregnancy Outcomes Reporting System (IDPH) has shown a statistical increase in the incidence of abdominal wall defects. In general rates for Chicago are lower than those for Illinois as a whole. This finding is consistent with the Regional Perinatal Centers perceived distribution of rural origin of abdominal wall defect cases. Congenital diaphragmatic hernia (CDH) cases are not significantly elevated in the state surveillance data, but do seem to be more common in the more rural regions. DCNPE has been reported to be a useful experimental teratogen in several animal models of CDH. Recent work from Europe suggests a teratogenic mechanism. Therefore, the initial work suggests the plausibility that a herbicide in common use may be a candidate teratogen and may explain the apparent rural distribution of an increasing trend of specific fetal anomalies in IL. A detailed case-control study and a correlation study of anomaly maps with state water quality are both justified, and required, to test the validity of our proposed hypothesis.

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First trimester ultrasound identification of severe congenital anomalies associated with Pallister-Hall syndrome.

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Introduction: First trimester ultrasound examination allows for early dysmorphic studies of fetuses, sometimes preceding invasive prenatal diagnosis.

Case report: This 23 yo mother previously had a 19 wk fetus with short-limb dwarfism, polydactyly and hypothalamic hamartoma. Short-limb polydactyly syndrome type II was suspected. Direct molecular testing was not available. In her second pregnancy, 12 week-ultrasound examination revealed significant shortening of all long bones. Fetopathologic exam confirmed these findings along with polydactyly and absent pituitary. Molecular testing for *GLI3* mutations is pending.

Conclusion: This severe form of Pallister-Hall syndrome has been described in neonates. This is the first documentation of first-trimester identification.

Non-random XCI and Second trimester RSA family maps to Xq28. *M.M. Thouin¹, C.A.G. Scacheri³, J.M. Giron¹, C. Romig², E.P. Hoffman^{1,2}.* 1) Children's National Medical Center, Washington, DC; 2) George Washington University, Washington, DC; 3) GeneDX, Rockville, MD.

Highly skewed X chromosome inactivation (XCI) occurs in 2% of women and involves greater than 90% of cells inactivating the same X. The etiology ranges from cancer and monozygotic twinning, to a carrier state for X-linked cell lethal trait. Pegoraro et al. (1997) demonstrated that highly skewed XCI patterns are heritable, and showed concordance of skewing between oral fibroblasts and lymphoblasts. In a recent case-control study (Lanasa et al, 2001), 14% of women experiencing recurrent first trimester pregnancy loss (RPL) displayed highly skewed XCI. Here we present a family ascertained with multiple second trimester known male losses, accompanied by polyhydramnios and ventriculomegaly. She and several maternal female relatives show highly skewed inactivation, preferentially activating their paternal X. Also of interest in this family is the non-concordance of skewing between blood lymphoblasts and oral fibroblasts, which show random XCI. Exclusion mapping was done using microsatellites throughout the X chromosome; this mapped the gene to a 50cM region in Xq28. This region includes several target genes of interest including arginine vasopressin receptor 2 (AVPR2), G6PD, and Factor 8. AVPR2 is expressed in the kidney tubule and helps maintain water homeostasis. Mutations are associated with nephrogenic diabetes insipidus (NDI), and polyhydramnios. Deficiencies in G6PD and Factor 8 both cause blood-related disorders and may explain the tissue-specific skewing in this family.

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A pairwise-splitting approximation to NPL linkage tests using the S_{pairs} scoring function and the linear model.

O.T. Atlason, D.F. Gudbjartsson, A. Kong. Statistics, deCODE genetics, Reykjavik, Iceland.

Working within the framework of nonparametric linkage as proposed by Kruglyak et al. (1996) we explore a method of approximating the NPL score in the special case of the scoring function S_{pairs} . We can approximate the contribution of each pair of affecteds, (**a**,**b**), by only regarding information contained in a sub-pedigree containing all paths between **a** and **b**. In the linear model introduced by Kong and Cox (1997), the NPL score is a sufficient statistic. We can thus approximate calibrated linkage tests for large pedigrees deterministically in the sense that there is no variation when the same data is analyzed more than once. Our approximation may also be used to do QTL analysis and is more accurate than the approximation proposed by Fulker et al. (1995).

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Test for Association in Family-based data. *X. Liu, D. Gordon.* Lab Statistical Genetics, Rockefeller Univ, New York, NY.

The transmission/disequilibrium test (TDT) (Spielman et al. 1993) can test for association between a genetic marker and disease susceptibility loci by using one affected child from affected sibships. Based on a TDT type linkage test developed by Spielman and Ewens (1996) for multiallelic markers, we propose a class of association tests for nuclear families with any number of affected children, for both diallelic and multiallelic markers. The association tests are constructed using the symmetry between the transmitted/nontransmitted alleles from heterozygous parents to affected children under linkage equilibrium. One of the proposed test statistics reduces to the TDT for nuclear families of single affected child, and to the association tests proposed by Martin et al. (1997) for affected sib pairs. The association test used in simulation and for real data (sitosterolemia) is the one which has the best overall power in detecting association. This association test is generally more powerful than the association test proposed by Martin et al.(1997). For the sitosterolemia data set, the association test has its most significant result (p -value=0.0012) for the marker locus on the same bacteria artificial chromosome as the disease locus.

Using Test of “Longest Run” to Estimate Region-Specific P-value in Gene Mapping Studies. *C.S.J. Fann¹, I.B. Lien², C.H.L. Chen¹, L.Y. Chen², C.J. Chang³.* 1) Epidemiology & Public Health, Inst Biomed Sci, Acad Sinica, Taipei, Taiwan; 2) Department of Mathematics, National Chang Hua University of Education, Taiwan; 3) Department of Medical Research, National Taiwan University Hospital, Taiwan.

In testing the association between a putative disease gene and genetic markers, one of the widely used study design is to focus on a pre-selected candidate region saturated with large number of single nucleotide polymorphisms (SNPs). In testing the significance, point-wise p-values need adjustment for multiple comparisons. Although it is known that problem of multiple comparison is less severe for tightly linked markers, it is essential to realize that improper correction inevitably lead to either increased false-positive rates or decreased statistical power. In this project, we studied the probability observing the longest run with fixed number of SNPs as the region-specific p-value. Longest run is defined as a consecutive string of positive signal from association tests. Previously, the asymptotic theory of “Longest Run” has been implied successfully in measuring the similarity between DNA sequences with sufficient length. In our case, however, the region might contain only a hundred SNPs; therefore we developed a method that calculates the exact distribution of the length of the longest run that also allows for a few random errors in the string. To account for the presence of linkage disequilibrium between tightly linked SNPs, Markov first order dependence was used to modify our method. The efficacy and validity of our methods are demonstrated by using simulated data.

Linkage disequilibrium error analysis via the application of bootstrap methods. *E.D. Kelly¹, R. McManus².* 1) Hitachi Dublin Laboratory, Hitachi Europe Limited, Dublin 2, Ireland; 2) Department of Clinical Medicine, Trinity College Dublin and Dublin Molecular Medicine Centre at St. James's Hospital, Dublin, Ireland.

In the hunt for the genes responsible for the more common, complex diseases such as cancer and heart disease, linkage disequilibrium testing has become a vital element. When the phase of sample data is known, Markov chain Monte Carlo (MCMC) methods may be used to estimate the level of disequilibrium between pairs of loci. However, if the haplotypic composition of patient data is unknown, MCMC methods are not applicable, and instead a likelihood ratio test is employed. Use of this method has become widespread, but despite this, there has been little investigation of the degree of error involved. This has been partially due to the significant extra computational demands that it places upon the resources of the investigator. We have developed an EM-based algorithm for the estimation of LD between locus pairs which incorporates an exact solver for SNP data and non-parametric bootstrapping for the calculation of accurate error bars. We present the results when LD is carried out on data derived from a population of cystic fibrosis patients. Initially the phase of the data is known and an MCMC method is used to calculate the level of LD between each locus pair. The phase of the data is then randomised and LD is recalculated using the EM-based likelihood ratio test. The technique of bootstrapping is then applied to generate error bars about the single point results. The accuracy of the EM method and the sensitivity of the bootstrap procedure to data sizes are assessed via a comparison between the results for the phase-known and the phase-unknown data. As expected, the accuracy of the estimation falls off as fewer individuals are included in the analysis. However, what is of particular note is the extent of the single standard deviation error bars. For statistically significant results (values of P less than 0.05), the error bars are small, with medians closely corresponding to the point estimates derived from the original data. In contrast, the error bars associated with non-significant point estimates ($P > 0.05$) are large, but importantly lie outside the 0.05 significance limit.

Path analysis of protein expression data in cancer-related metabolic pathways. *D.J. Kaufman, K. Buetow.* Lab of Population Genetics, NCI, Gaithersburg, MD.

Algorithms used to analyze gene expression data from microarray experiments often focus on differences between single genes, or group genes by patterns of expression rather than by known biological relationships between the gene products. To examine known biological relationships between gene products in well-studied metabolic pathways, we applied path analysis to cDNA microarray data. Path analysis is a statistical technique that identifies the correlation structure that best explains relationships of various proteins in a metabolic pathway, to determine which proteins in a stream most influence expression of downstream genes. Here, eight cancer-related pathways (including ones related to cell cycle regulation, growth and differentiation, apoptosis signaling, and immune/stress response), were examined in 60 cancer cell lines previously characterized by the NCI. The lineages were analyzed in aggregate to characterize correlations common to different tumor types. For example, in the G1/S checkpoint pathway, phosphorylated Rb permits transcription of S-phase genes. In the NCI cell lines proteins (cyclin D, cyclin E, cdk2, cdk4, cdk6) directly upstream of Rb did not influence Rb levels. The influence of contact inhibition and DNA damage (through p27, p21 and p53) remain intact but were only slightly correlated with Rb. In contrast, TGF β levels further upstream accounted for 25% of variation in Rb expression. Such analyses will become more powerful when they are used to compare the structure and magnitude of correlations in diseased and normal tissue. We will use similar path analysis to compare the best-fitting models of lung tumor and normal lung tissue. We are also developing public domain software for distribution to perform simple path analyses.

Program Nr: 2336 from 2002 ASHG Annual Meeting

Unbiased Quantitative Population Association Test. *J. Zhao, M. Xiong.* Human Genetics Ctr, Univ Texas, Houston, TX 77030.

In the past several years, there have been increasing interests in the genetic studies of common diseases. It is now recognized that linkage analyses which have been successfully applied to genetic studies of simple Mendelian diseases have been much less successful. Population association studies have been proving to be an alternative to linkage analyses for mapping complex disease genes when a dense map of SNP markers is available. However, population substructure creates spurious association, which increases type 1 error and compromises the performance of population association studies. To overcome this problem, various statistical methods for control of population stratification when analyzing case-control studies have been proposed. Although genomic control methods for association studies can alleviate spurious association, they need to genotype a large number of additional markers. In this report, we propose an unbiased population association test for identification of quantitative trait loci using unrelated individuals. We will show that in the presence of population substructure the proposed test is still valid and will not increase the type 1 error. Furthermore, the proposed test does not need to genotype additional markers. We evaluate the performance of the proposed test statistic through both asymptotic analytic analyses and simulations. Our preliminary results show that the proposed test has higher power than several genomic control methods.

Program Nr: 2337 from 2002 ASHG Annual Meeting

Assessing the Accuracy of Algorithmic Haplotype Inference. *A. Windemuth, B.A. Salisbury, R.S. Judson, J.C. Stephens.* Genaissance Pharmaceuticals, New Haven, CT.

Theoretical arguments and empirical data both justify the utilization of algorithmic approaches to inferring haplotypes from population-based genotypic data. First, under a simple but realistic model of molecular evolution, it can be shown that the pair of haplotypes that explains an individual's multi-site genotype will often be unique. Therefore, algorithms that deduce haplotypes from multi-site genotypic data are likely to be very accurate unless there are serious departures from the model. The model is one in which recombination and recurrent mutation are very limited. Second, considerable evidence is accumulating that recombination rate heterogeneity has produced long regions, 10s of kb if not longer, within which little or no evidence of recombination is seen, even from large population samples. SNPs within such regions can be used for inferring the haplotype structure relatively easily. Third, we have tested our own statistical method of haplotype inference on an empirical sample of over 2600 genes. The method achieved 97% agreement with haplotypes that were deduced strictly according to the rules of Mendelian inheritance in two families totaling 17 individuals; this high level of accuracy is comparable to what can be achieved by the best molecular methods.

Methods and software for association tests of uncertain haplotypes in case-parent trios. *F. Dudbridge*. UK HGMP Resource Centre, Cambridge, UK.

Association testing is used to fine map genes through linkage disequilibrium and to assess the trait association of candidate genes. Haplotype analysis can be useful when an ancestral chromosome is better marked by several closely linked markers than by any one marker, or when there are aetiological haplotype effects. Haplotype methods are also necessary for linear modelling methods which account for disequilibrium with primary linked loci. These methods are usually implemented by likelihood ratio tests. In the case-parents design, the likelihood consists of a parental component and an offspring component conditional on the parents. When the haplotypes are certain, the conditional part is independent of the population structure and yields the transmission/disequilibrium test. For diploid genotype data, some haplotypes are uncertain and standard theory does not apply to the conditional likelihood. The full likelihood can be used with an E-M algorithm which estimates parental haplotype frequencies under both null and alternative hypotheses. This approach is sensitive to population structure, association in the absence of linkage, ascertainment criteria, and patterns of missing genotypes; but these factors result in a misspecification of the parental frequency model and do not affect the matching of cases to controls. When the null is that no haplotypes are associated, a permutation test is possible by swapping the transmission status of haplotypes, retaining the same status for all possibilities in uncertain cases. The "unphased" software package implements association tests which include uncertain haplotypes and missing genotypes, includes a permutation test, and implements unbiased TDT for the certain haplotype cases. Main effects and full interaction tests are available to account for primary linked loci, and tests of individual haplotypes and pairwise comparisons are possible. The tests are also implemented for unrelated subjects and a graphical user interface is available on the world wide web.

Program Nr: 2339 from 2002 ASHG Annual Meeting

Software for Simulation Studies of Complex Traits: SIMLA. *M.P. Bass, E.R. Martin, E.R. Hauser.* Center for Human Genetics, Duke Univ Medical Ctr, Durham, NC.

We have developed a software package to assist with genetic analysis of complex traits. SIMLA is a general use simulation package that can simulate under specific linkage and association scenarios for dichotomous traits in nuclear and extended families. This package allows for simulation of variable levels of linkage and allelic association between the markers as well as between markers and one or more disease loci. We have used this software to examine correlation in linkage and family-based association statistics (Martin et al. 2001), to evaluate a new version of the PDT for genotypic association (Martin et al. 2002), and to examine the power of the Ordered Subset Analysis (OSA) to detect genetic heterogeneity (Hauser et al. 2001). We are in the process of generalizing SIMLA to simulate from observed family structures and from observed marker maps to allow for empirical p-value calculation for observed lod scores. We will describe how to obtain the software, show examples of the input and output and provide usage documentation.

Haplotype Analysis of Quantitative Phenotypes: Methods and Application to Adrenergic Receptor

Polymorphisms and Complex Cardiovascular Traits. *A. Chandra*¹, *B.K. Rana*¹, *A. Tahgva*², *D. Fallin*², *N.J. Schork*¹. 1) Polymorphism Resesarch Laboratory; Psychiatry, University of Calif, San Diego, La Jolla, CA; 2) Epidemiology, Bloomberg School of Public Health, Johns Hopkins University.

Recent developments in high-throughput genotyping technologies have enabled researchers to identify numerous polymorphic sites within candidate genes that may influence common complex disease and traits. Making use of these technologies is complicated, however. When two or more polymorphic sites are identified within a gene in unrelated individuals, tedious molecular techniques are necessary to determine the actual haplotype of each individual. Current statistical strategies are limited to estimating haplotype frequencies in the population or to only inferring individual haplotypes based on homozygosity. Since there may exist multiple haplotype combinations in a population sample, it is often difficult to assign individual haplotypes, especially in order to analyze haplotype effects on a quantitative phenotype. We have developed a mixture modeling method for analyzing haplotype effects on a quantitative phenotype using individuals sampled randomly from the population at large. We have applied this method to analyze SNP data on two highly polymorphic beta-adrenergic receptor loci, *ADRB1* and *ADRB2*, and to demonstrate haplotype effects on various complex cardiovascular traits including systolic and diastolic blood pressure, and adrenergic receptor cell surface density. Our methods will help facilitate mapping and sequencing efforts and allow genetic analysis of complex quantitative phenotypes that make use of this resource.

Comparing haplotyping program performance: Accuracy of haplotyping with polymorphic markers and SNPs.

E.M. Lindholm¹, M. Durner¹, D.A. Greenberg^{1,2}. 1) Div Stat Genet, Columbia Univ, New York, NY; 2) Div Clin Genet Epi, NY Psych Inst. New York, NY.

Genehunter (GH) and Simwalk (SW) are two programs commonly used to determine haplotypes. We tested the accuracy of these programs by investigating the frequency of haplotyping errors in tightly linked markers with varying informativeness. We generated marker data on 10 markers with no recombination. The number of alleles was 2-9 with equal allele frequency at each marker except for SNPs, in which we tested three allele frequencies (0.5, 0.25 and 0.1 for the minor allele). We generated 200 nuclear families of varying size following a population based distribution. The results are summarized in the table. Overall, the frequencies of haplotyping errors was low (1-2%) for markers of alleles greater than 3. In biallelic markers with 0.5 allele frequency, GH inferred wrong haplotypes in almost 10% of the families. Using SW the same estimate was 6%. In the wrong haplotypes, the average number of incorrect markers was about 1 for markers with 4-9 alleles; with 3 or 2 alleles/marker, about 2 markers were wrong. Among 2000 family analyses total, 78 had incorrect haplotypes. Of the 78, 34 errors were identical in GH and SW while 26 were wrong in GH only and 19 in SW only. [Support: NIH Grant DK31775, NS27941, MH48858]

Alleles	9	8	7	6	5	4	3	2	2	2
Freq.								0.5	0.25	0.1
SW errs	0.5	1	3	1.5	2	1.5	5.5	6	5.5	0
GH errs	1.5	2	3	1	1.5	2	6	9.5	4	1.5

Program Nr: 2342 from 2002 ASHG Annual Meeting

Haplotyping with respect to linkage disequilibrium on empirical data. *S. Lin, DJ. Cutler, A. Chakravarti.*
McKusick-Nathan Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.

Contemporary sequencing and genotyping methods do not provide information on linkage phase in diploid organisms. The application of statistical methods to samples of diploid sequences is a potentially time and labor saving method by which to reconstruct the linkage phase of many polymorphic sites. Stephens et al. (2001) devised one such method, which incorporates concepts from population genetics theory, in a markov chain monte carlo algorithm.

We modified and improved the algorithm, accounting for missing data, and applied the resultant computer program to empirically derived, phase-known sequences. The sequences comprise eight X-linked regions from a sampling of forty males (Cutler et al. 2001) and range from 87 to 327 kb in length with 45 to 165 segregating sites. 100 random pairings of the set of forty haplotypes, to create diploid genotypes of known phase, for each region was run to determine the accuracy of the program. We find that phase reconstructions by the modified program are highly accurate over regions with high linkage disequilibrium (LD). If only high frequency polymorphisms ($q > 0.2$) are examined, reconstructions are 95.2% accurate over entire 100 kb stretches and 98.6% accurate within blocks of high LD (defined as relative LD > 80%).

With portions of the computer program coding the estimation of phase call certainties excluded, the program runs with remarkable speed. For, example, on standard 800 MHz PC machines, 10,000 iterations of the algorithm on a specific locus with 165 segregating sites takes no more than ten seconds.

Cutler DJ, Zwick ME, Carrasquillo MM, Yohn CT, Tobin KP, Kashuk C, Mathews DJ, Shah NA, Eichler EE, Warrington JA, Chakravarti A (2001) High-throughput variation detection and genotyping using microarrays. *Genome Res* 11:1913-25

Stephens M, Smith NJ, Donnelly P (2001) A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 68:978-89.

PEDMST, A New Program for Identity Coefficients Calculation and IBD Patterns Enumeration. *C. Zhu¹, J. Graham²*. 1) Computing Science, Simon Fraser University, Burnaby, BC, Canada; 2) Statistica and Actuarial Science, Simon Fraser University, Burnaby, BC, Canada.

Since currently no specific model has been built to list all the possible IBD patterns between the genes from the individuals defined by a pedigree at certain autosomal locus, we design a labeled tree structure to enumerate those patterns, and also develop a recursive algorithm to construct the tree with one basic tree expanding rule and seven constraint rules. The final output patterns, excluding impossible ones given the relationship of the individuals in the pedigree, can be counted and listed using path searching on the constructed labeled tree. PEDMST (short name for Pedigree Master), written in Visual C++6.0, is the program where we implement this idea. Totally there are three major functionalities. First, the function of pedigree operation allows user to construct the pedigree or import the pedigree from the text file used by the other software such as PFIDDLER. A special graph drawing algorithm based on the generations of the individuals is designed and implemented for importation. Second, the function of kinship and identity coefficients calculation gives the user not only the numerical result but also the graphic result in terms of detailed or condensed identity states. Generalized kinship coefficients are used to compute the identity coefficients. Third, the function of IBD pattern enumeration allows the user to view all the possible IBD patterns between the input genes whose maternal or paternal source is specifiable. The maximal number of the genes currently is set to 10, but can be expanded easily later according to the large capacity of the tree model we use. PEDMST has received positive user feedback in functionality, usability, and visualization with its features such as the unique function of IBD pattern enumeration, the instant view of pedigree while editing, the graphic output of the calculation result, the capability of representing the pedigree and doing the computing on the same screen.

Program Nr: 2344 from 2002 ASHG Annual Meeting

Performance of Parallel Algorithms in Bioinformatics. *F. Sievers, E. Kelly.* Hitachi Dublin Laboratory, O'Reilly Institute, Trinity College, Dublin 2, Ireland.

Using a bootstrapped Haplotype Frequency Estimation Algorithm as an example we demonstrate the importance of parallel computing in Bioinformatics, as the calculation time for many problems in Bioinformatics scale exponentially with the number of loci and/or patients. This is particularly important when determining confidence intervals. We show that a naive equi-partition parallelisation scheme does not scale well, and better load-leveling of resources can be achieved using a Master/Slave approach. This is because of the long tail of the distribution of calculation times for different bootstrap-populations. These bootstrap times are the sum of individual calculation times for different initial conditions which in turn exhibit a great variability. We explain how performance can be enhanced by redundant parallel computation but that the accuracy of results could be degraded by this approach. We show how all those considerations are affected by Linkage- and Hardy-Weinberg Dis/Equilibrium. We address how marginal frequencies for different bootstrap-populations impact on the variability of the computation times.

PowerTrim: An Automated Decision Support Algorithm for Preprocessing Linkage Data. *T.A. Thornton^{1,2}, J.L. Haines²*. 1) Center for Integrative and Cognitive Neuroscience, Department of Biomedical Informatics, Vanderbilt University; 2) Program in Human Genetics, Vanderbilt University Medical Center, Nashville, TN.

Statistical genetics software packages for linkage analysis have unique pedigree size and structure constraints. As a result, analysts are often forced to exclude data from some individuals in a given family. The choice of which subjects to remove may involve consideration of numerous rules based on basic principles of inheritance. In addition, the task of locating transcriptional or data-entry errors, which can result in unsuccessful or erroneous analysis, is very time-consuming. We designed an algorithm called PowerTrim, coded in Perl, whose goals were to: 1) identify and report errors in pedigree data in a user-friendly manner; 2) trim from the pedigree those individuals who provide no information; 3) recommend how a pedigree might be further trimmed to maximize the power of the linkage analysis while conforming to the constraints of the target software; 4) automate the production of edited data and pedigree files. Toward the third goal, PowerTrim iteratively examines the relative contribution of each individual within each family to the power of the linkage analysis, using information on data-completeness and familial relation. For example, it reports the number and percentage of markers genotyped by individual and by family and allows batch-trimming of individuals within some or all families who fall below a user-specified threshold for data-completeness. PowerTrim also provides interactive decision support, offering recommendations based on known data constraints of the GENEHUNTER-Plus and Allegro programs. Each of these programs sets different limits on the number of bits the pedigree may contain, recognizing that the space and time complexity of the analysis increases exponentially with the size of the pedigree. Existing options in these programs are not interactive or user-directed and make arbitrary choices. PowerTrim calculates the pedigree bit size and then allows the user to choose which individuals to trim, while updating and reporting on which pedigrees continue to exceed the limits of either GENEHUNTER-Plus or Allegro.

Program Nr: 2346 from 2002 ASHG Annual Meeting

Population substructure effects on the extent of genotype sharing: A tool for detecting the presence of relatives in databases. *K.M. Teshima, H.S. Lee, R. Chakraborty.* Center for Genome Information, Department of Environmental Health, Univ. Cincinnati, Cincinnati, OH.

The random match probability in a substructured population is larger than that in a single homogeneous population. When each individual in a database is typed for the same set of loci, the distribution of the number of loci showing identical genotypes in pairwise comparison of profiles offers information as to the extent of population substructuring. Computations based on coalescence theory of mutation drift balance show that the mean number of loci exhibiting random match of genotypes increases with the coefficient of co-ancestry. However, the expected number of pairs with identical genotypes decreases with the number of loci at which identical genotypes are seen. Presence of relatives disturbs this monotonic relationship. Analytical results based on the Identity By Descent (IBD) theory, superimposed on the coalescence models of the sampling theory of allelic distribution, show that the distribution of loci showing genotype matches can be a tool for studying presence of relatives in databases, in addition to it being an indicator of population substructure effects. In the DNA forensic context, this implies that a substantial frequency of partial DNA profile matches may exist in databases, in spite of each specific multilocus DNA profile being rare, in particular when databases are large in size. This theory is also applicable for detecting cryptic relatedness differences of cases and controls in population-based disease-gene association studies. (Research supported by US Public Health Service Research grants from the US National Institutes of Health).

80% Posterior Probability of Linkage of Panic Disorder to Small Region on Chromosome 7. *M.W. Logue^{1,2}, V.J. Vieland^{1,2}, R.J. Goedken¹, R.R. Crowe²*. 1) Biostatistics, Division of Statistical Genetics University of Iowa, Iowa City, IA; 2) Psychiatry, University of Iowa, Iowa City, IA.

This work represents a re-analysis of 390 autosomal markers from the Crowe et al. (2001) genome screen for panic disorder. Increased computing capacity allows us to use a version of the 2-point posterior probability of linkage, or PPL, statistic (Vieland, 1998) which removes the need to fix the genetic model by integrating the parameters of the genetic model out of the likelihood. The result is a statistic which is "model-free" and yet uses the information from all of the individuals in large pedigrees. The model integrated PPL reaches 80% on chromosome 7, 24% on chromosome 16, and is less than 12% across the rest of the genome. One advantage of the PPL is that it can be directly interpreted as a measure of the probability that there is a disease gene linked to a given marker. Even so, null simulations were performed and model integrated PPLs were computed for 10,000 simulated, unlinked markers. Only one null replicate exceeded the PPL of 80% on chromosome 7, and only 9 were greater than the 24% on marker 16. The model integrated PPL analysis provides a much clearer indication of a gene for panic disorder on chromosome 7 than previously achieved, although the localization properties of this method are still under investigation.

Program Nr: 2348 from 2002 ASHG Annual Meeting

Efficient simulation of p-values for linkage analysis. *K.K. Song¹, D.E. Weeks¹, E. Sobel², E. Feingold¹*. 1) Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA; 2) Dept Human Genetics, UCLA, Los Angeles, CA.

In many linkage studies, p-values are obtained through simulation since the underlying distribution of the statistic is unknown. However, this can be very computationally intensive, in part because the linkage statistics are sums of contributions from the different pedigrees. A "bootstrap" approach has been suggested (Terwilliger and Ott 1992, Leal and Ott 1993) that generates p-values more efficiently by resampling from the simulated data for each pedigree. The bootstrap idea has been successfully applied, but has never been theoretically studied. An entirely different method for increasing the efficiency of p-value simulation is Besag and Cliffords sequential sampling method (1991), but this method has never been applied to linkage analysis. We propose an algorithm to combine Besag and Cliffords method with the bootstrap method. We also derive variance expressions for several different p-value estimators, and use these to make practical recommendations about what estimators are best and precisely how to implement them.

Score statistics for mapping quantitative trait loci with extended pedigrees. *K. Wang.* Dept Biostat Div Stat Genet, Univ Iowa, Iowa City, IA.

The method of variance components is the method of choice for mapping quantitative trait loci (QTLs) with general pedigrees. Using a likelihood ratio statistic, this method can be computation intensive even for nuclear families. Here two score statistics to detect QTLs are derived, one assumes the dominance effect of the trait at the candidate locus is negligible, and the other one does not make such assumption. One prominent feature of these two score statistics is that they are very easy to compute. The approach here is asymptotically equivalent to the method of variance components, but is different in two aspects: First, it is based on a different analysis model; Second, it is based on the concept of separation of the segregation parameters, the parameters that describe the distribution of the phenotypic values in the population, and the linkage parameters, the parameters that measure the effect of the candidate locus on the phenotypic values. The separation of the model parameters greatly reduces the number of parameters to be dealt with in the analysis of linkage. The asymptotic distributions of both score statistics are derived. Simulation studies indicate that, compared to the method of variance components, both score statistics have comparable or higher power and their false-positive rates are closer to the respective nominal significance levels.

Effect of Winsorization on Power and Type 1 Error of Variance Components and Related Methods of QTL

Detection. *S.S. Shete*¹, *T.M. Beasley*², *C.J. Etzel*¹, *J.R. Fernandez*^{2,3,4}, *J. Chen*¹, *D.B. Allison*^{2,3,4}, *C.I. Amos*¹. 1) Dept Epidemiology, Univ Texas MD Anderson CA Ctr, Houston, TX; 2) Dept Biostatistics, Section on Statistical Genetics, Univ of Alabama at Birmingham, Birmingham, AL; 3) Dept of Nutrition Sciences, Division of Physiology and Metabolism, Univ of Alabama at Birmingham, Birmingham, AL; 4) Clinical Nutrition Research Center, Univ of Alabama at Birmingham, Birmingham, AL.

Variance components analysis provides an efficient method for performing linkage analysis for quantitative traits. However, power and type 1 error of variance components based likelihood ratio testing may be affected when phenotypic data are non-normally distributed (especially with high values of kurtosis) and there is moderate to high correlation among the siblings. Winsorization can reduce the effect of outliers on statistical analyses. Herein, we considered the effect of winsorization on variance-components based tests. We considered the likelihood ratio test (LRT), the Wald test and some robust versions based on the variance components method and we compared these tests with Haseman-Elston least squares-based tests. We found that power to detect linkage is significantly increased after winsorization of the phenotypes. Winsorization does not greatly diminish the type 1 error for the variance components based tests for markedly non-normal data. The robust LRT showed the best power for non-normal data. Finally, phenotype winsorization reduces the bias in estimation of the major gene variance component.

Selecting Genetic Markers for Association Analyses Using LD and Haplotypes. *Z. Meng^{1,2}, D.V. Zaykin², M. Mosteller², C. Xu², M. Wagner², M.G. Ehm².* 1) Bioinformatics Research Center, North Carolina State University; 2) Discovery Genetics, GlaxoSmithKline.

Researchers are discovering that selecting and genotyping SNPs (single nucleotide polymorphisms) at densities less than 100 kb can result in a large number of highly correlated SNPs. High-density SNP data have been used to illustrate that large regions of the genome exist with extensive linkage disequilibrium (LD) and others with less LD. Furthermore, the extent of LD drives the number of possible frequent haplotypes observed in the region. Several studies have illustrated that it may be possible to select SNPs to maximize the information obtained about a genomic region reducing the genotyping cost and simplifying the analysis. Methods are needed to select a minimum subset of SNPs sufficient to retain most of the information provided by haplotypes observed in the region. We extend David Clayton's htSNP selection method, which utilizes haplotype information and propose another method. Both procedures require genotype information on a small number of samples to select SNPs that would be typed on larger numbers of samples. We have studied the properties of these methods using simulated data sets in linkage equilibrium and disequilibrium to assess performance as measured by the proportion of rejected SNPs, as well as by changes in haplotype numbers and diversity. In addition, we have applied the methods to data sets with 368 and 137 samples with SNPs typed at average densities of 20 and 50 kb, respectively. The amount of genotyping can be reduced while maintaining the genetic information content throughout the regions.

On detection of population growth based on SNP data. *A. Polanski, M. Kimmel.* Department of Statistics, Rice University, Houston, TX.

Single Nucleotide Polymorphisms (SNP) are promising genetic markers due to their high density in human genome. SNP data have already been used in association studies of complex diseases; it is believed that eventually they will enable creating fine genetic maps for complex traits analysis. Researches also started using SNP data in population genetics models, for inference on demographic parameters and history. These researches included estimation of the product parameter of effective population size and mutation rate in the population of constant size under hypotheses of spatial (chromosomal) distributions of SNPs, and estimation of parameters of exponential or stepwise models for populations growth.

In this study we address the following problem: How reliable and accurate are the estimates of population growth parameters, based on SNP data? This problem has not been fully explored in the existing literature. Using statistical simulations we estimate distributions and confidence regions of estimates of parameters of populations growth. In our simulations we used newly developed expressions for distributions of coalescence times in time - varying population size evolution, which greatly improved efficiency of numerical computations. Sample size ($n=20$) and number of SNP sites ($K=50$) which we used in our simulations, are comparable to those reported in observational studies.

The obtained distributions of maximum likelihood estimates of the parameter of the exponential growth scenario have atoms at zero and long right tails. For parameters of the stepwise growth scenario, loglikelihood functions have very long ridges (over many decades of the scale) of almost the same value of log likelihood. Therefore parameters of stepwise change cannot be obtained by maximization of the loglikelihood. Instead (like in other studies) we were only able to determine the shapes of regions, which correspond to high likelihoods of data. We have also analyzed observational data on SNP frequencies reported in the literature. When taking into account confidence regions for parameters obtained in our simulations, these data are not inconsistent with the hypothesis of population growth.

The impact of population stratification in case-control studies. *M.F. Freedman**^{1,2}, *D.E. Reich**¹, *S.B. Gabriel*¹, *P.M. Sklar*^{1,2}, *J.N. Hirschhorn*¹, *D. Altshuler*^{1,2}. 1) Whitehead Inst., Cambridge; 2) Mass. General Hospital, Boston.

*The first two authors contributed equally

Case-control studies are the most powerful method for identifying genes contributing to common human disease. There has been extensive debate, however, about the extent to which population stratification (systematic differences in ancestry between cases and controls) can cause false-positive associations.

To assess whether stratification occurs in real studies, we selected 81 unlinked SNPs, and genotyped them in >200 samples from each of 7 studies.

One study showed significant evidence for stratification according to a test developed by Pritchard & Rosenberg (1999). A computer program (Pritchard 2000) was then used to identify the samples contributing to the structure (a few African Americans in a study originally thought to include only Caucasians). Thus, Pritchard's suite of methods is highly reliable at dealing with stratification due to large differences in ancestry.

Finer degrees of stratification, however--which might arise due to individuals of both north and south European ancestry being included in the same study--cannot be practically addressed by these methods without genotyping thousands of SNPs. To set a maximum on the amount of 'cryptic' stratification consistent with our data, we therefore turned to Genomic Control methods (Reich & Goldstein 2001; Devlin & Roeder 1999). The analysis showed that 6 of 7 studies were consistent with stratification at a level that would produce many false-positive associations in a study involving thousands of samples.

Thus, while Pritchard's methods are adequate for correcting for gross stratification, they cannot rule out cryptic stratification. GC methods, however, provide a way of not only measuring, but also correcting for any stratification that exists. Application of GC allows investigators to be confident that any associations that are detected, and also pass the GC test, are real. We recommend that the GC test be used as a minimum criterion for publishing associations in all future case-control studies.

Sample sizes for detecting SNP variants in common diseases. *S.E. Hodge¹, S.L. Slager^{1,2}, R. Allikmets¹, T.C. Gilliam¹.* 1) Columbia U., New York, NY; 2) Mayo Clinic, Rochester, MN.

We examine a strategy to evaluate SNP variants within a gene, or across networks of interacting genes, that confer susceptibility for a complex trait, particularly in the presence of allelic or locus heterogeneity. The basic concept is to look for differences in total number of SNPs between cases and controls. Assuming Hardy-Weinberg equilibrium, and independence between SNP sites, we derive sample-size formulas as a function of desired type I (α) and type II ($\beta = 1 - \text{power}$) errors and of n = average no. of neutral polymorphisms/haplotype: $n = [Z(\alpha) + Z(\beta)]^2 (4n+1)$, where n = no. of controls = no. of cases, and $Z(x)$ = standard normal deviate with area of x in the right tail. If there is locus heterogeneity, with only a proportion p of cases having a variant in this particular gene, $(4n+1)$ is replaced by $(4n+p)/p^2$. The table shows values of n for selected values of n , p , and α , and for power=80%.

$\alpha=.05$	$p=1.0$	$p=0.5$		$\alpha=.10$	$p=1.0$	$p=0.5$
$n=0.5$	19	64		$n=0.5$	14	47
$n=1.33$	40	147		$n=1.33$	29	107

We also derive sample-size formulas for when an investigator desires unequal numbers of cases and controls; also how to *choose* unequal numbers so as to minimize cost and maximize power. Finally, we illustrate the method with ABCR gene data from 150 Stargardts (STGD) cases, 166 AMD (age-related macular degeneration) cases, and 220 controls. We observed 249 variants among 2,046,300 sites in STGD vs. 103 among 3,001,240 in controls, yielding a significant difference ($P < .00001$). For AMD, the difference was significant ($P < .00001$) or not, depending on whether certain common SNP variants were removed or included. This approach should prove helpful in detecting susceptibility SNP variants in complex diseases.

Program Nr: 2355 from 2002 ASHG Annual Meeting

Power computations with misclassified and missing genotype data in association studies of nuclear families. *R.W. Morris, N.L. Kaplan.* Biostatistics Branch, NIEHS, Res Triangle Park, NC.

We study power loss due to genotype errors and missing observations for a family-based likelihood ratio test for association. Under a general model of genotype misclassification or when some, but not all, parents are missing, we employ an EM algorithm to compute the noncentrality parameter under a specified alternative for a two-degree of freedom chi-square distributed test statistic. For power loss characterized by test efficiency, determined as the ratio of the noncentrality parameter for data with misclassified or missing observations to the noncentrality parameter for perfect or complete data, we find that high levels of genotype misclassification can result in substantial decline in test efficiency. With partial missing parental data, however, test efficiency does not decline greatly until most families are missing one or both parents. When both misclassification and missing genotype data occur together, we find the reduction in test efficiency can be substantial.

Two-stage design for tests of gene-environment interaction using haplotype. *X. Liu, D. Fallin.* Genetic Epidemiology, Johns Hopkins University, Bloomberg School of Public Health, Baltimore, MD.

Haplotype-based methods for association analyses have received increasing attention given the ability to capture LD patterns in a more informative way than single polymorphisms. With the advent of high-throughput SNP genotyping, it is now possible to gather genotype data on 100s-1000s of SNPs for large case-control or family-based projects, enabling haplotype analyses at hundreds of candidate genes and potentially across the genome. With this in mind, methods for data reduction and interpretation of such a large number of data points are needed. For projects with a primary interest in gene-environment interaction, such as pharmacogenetic studies, we propose a two-stage design based on haplotypes to provide a powerful and efficient strategy for such data reduction and interpretation: Case-only haplotype analysis followed by a full case-control or trio-based analysis. The first step involves a powerful case-only approach to prioritize regions for subsequent analysis. We show the improved power of this approach, but call attention to the potential bias when the gene region and environmental exposure are correlated in the general population, increasing the false positive rate. To maintain an appropriate overall type 1 error, we propose a subsequent step using a case-control or case-trio design, which is robust to the background GxE correlation bias. We suggest step 1 and step 2 threshold levels to achieve a desired overall type 1 error and show the utility of this triaged design versus a single step analysis requiring the genotyping of all samples on all candidates. This two-stage design using a highly sensitive (high power) GxE test followed by a more specific (correct type 1 error) case-control analyses could provide an efficient way to analyze genome-wide information to identify regions or candidate genes that modify exposure or treatment effects on disease outcomes.

Application of the False Discovery Rate to genome-wide significance thresholds in association analyses. *S.K. Service¹, N.B. Freimer¹, C. Sabatti²*. 1) Neurobehavioral Genetics, UCLA, Los Angeles, CA; 2) Statistics Department, UCLA, Los Angeles, CA.

The mapping of complex traits using association analyses in population samples will likely require the testing of thousands of microsatellite or SNP markers. Unlike linkage analyses, association analyses do not have clear guidelines for significance thresholds that appropriately deal with multiple testing issues. Deciding on an appropriate threshold is further complicated by dependence between marker loci, the level of which varies across the genome. We explore the conceptual and practical implications of the multiple testing procedure known as the False Discovery Rate (FDR) in the area of association mapping for complex traits. Using simulated data, with varying power to detect association and varying degrees of dependency between markers, we show that the FDR correction has consistently higher probability to detect true signals than does the traditional Bonferroni method. This increase in power is most pronounced when attempting to identify multiple loci, as might be expected in complex trait mapping, and we show FDR to be up to twice as powerful as the Bonferroni method when we stipulate that multiple loci are to be found. As might be expected, the trade-off is in the absolute number of false positives. The FDR is designed to control the ratio of false positive rejections to total rejections, therefore while the number of false rejections in our simulations using FDR was higher than that found using Bonferroni, the proportion of false rejections was controlled, and we show this to be true even in the case of strong dependency between markers. While FDR's power is higher than Bonferroni in all scenarios we have examined, both methods suffer from decreased power when markers are highly dependent, highlighting the need for developing resampling based rules to control the FDR.

Genetic analysis of ordinal traits. *H. Zhang*. Dept Epidemiology/Public Hlth, Yale Univ Sch Medicine, New Haven, CT.

Many health conditions including cancer and psychiatric disorders have a complex genetic basis, and genes and environmental factors are likely to interact one another in the presence and severity of these conditions. Assessing familial aggregation and inheritability of disease is a classic topic of genetic epidemiology, which is commonly referred to as segregation analysis. While it is routine now to conduct such analyses for quantitative and dichotomous traits, there do not exist methods and software that accommodate ordinal traits. To this end, I will present a latent variable model. The advantage of this latent variable model lies in its flexibility to include environmental factors (usually represented by covariates) and its potential to allow gene-environment interactions. The model building employs the EM algorithm for maximization and a peeling algorithm for computational efficiency. Asymptotic theory has been established for statistical inference and simulation studies have been conducted to confirm that the asymptotic theory is adequate in practical applications. This model is applied to examine the familial transmission of alcoholism, which is categorized into three ordinal levels: normal control, alcohol abuse, and alcohol dependence. Not only does our analysis confirm that alcoholism is familial, but also it suggests that the transmission may have a major gene component which was not revealed by previous analyses using dichotomous traits. Potential use of our latent variable for linkage analysis of ordinal traits will also be discussed.

Methods for testing familial aggregation of diseases in population-based samples: Application to lymphoproliferative cancers in Swedish registry data. *R.M. Pfeiffer¹, M. Gail¹, K. Hemminki², L.R. Goldin³*. 1) Biostatistics Branch, DCEG, National Cancer Institute, Bethesda, MD; 2) Dept. of Biosciences at Novum, Karolinska Institute, Huddinge, Sweden; 3) Genetic Epidemiology Branch, DCEG, National Cancer Institute, Bethesda, MD.

Familial aggregation of diseases can be tested in case-control or cohort studies. A unique cohort for conducting such tests is the Swedish Family Cancer Database (Hemminki et al. *Acta Oncol* 40:772, 2001) which includes 10.2 million individuals with defined familial relationships, over 1 million of whom have a confirmed cancer diagnosis. From this database, we have selected relatives of all cases diagnosed with a lymphoproliferative cancer along with relatives of matched controls. We propose a survival analysis method for testing familial aggregation comparing disease occurrence in families that were ascertained through a case proband with disease occurrence in families that were ascertained through a control proband. We outline the method that extends work presented by Liang (*Genet Epidemiol* 8:329,1991). Let t_{ij} denote the age or age at onset of disease for member j in family i . The parameter, t , is modeled by a marginal Cox model $l(t_{ij} | X_{ij}, Z_{ij}) = l_0(t_{ij}) \exp(b X_{ij} + g Z_{ij})$, where l_0 denotes the arbitrary baseline hazard function, X_{ij} stands for measured covariates for individual j in family i , for example gender and decade of birth, and Z_{ij} is an indicator of the proband's disease status, i.e. $Z_{ij} = 1$ if the proband of family i is a case and 0 otherwise. Testing for familial aggregation corresponds to testing $H_0: g = 0$. The parameters are estimated under a "working independence" assumption, accounting for truncation arising from the database design. We use the robust sandwich covariance matrix to account for the dependence of the family members. This can be compared to analysis of observed versus expected cancers in first-degree relatives of case-probands based on external population rates. Families of Hodgkin's disease cases and controls are used as an example to illustrate the method.

Haplotype Linkage Disequilibrium Mapping of Quantitative Trait Loci. *R. Fan*¹, *M. Xiong*². 1) Dept of Statistics, Texas A&M Univ, College Station, TX; 2) Human Genetics Center, University of Texas-Houston, Houston, Texas.

In this report, a mixture model is proposed for high resolution multipoint/haplotype linkage disequilibrium mapping of quantitative trait loci (QTL). Suppose that a quantitative trait locus is located in a chromosome region, in which several markers such as SNPs are typed. Since the trait locus is not necessarily at a typed marker locus, the genotype at the trait locus is unknown. By using information of markers/haplotypes in the chromosome region, the conditional probability of the genotypes at the trait locus given the marker/haplotype information can be calculated. Then a mixture model can be constructed under the normal assumption. The maximum likelihood method can be used to estimate the contribution of a putative QTL within the region. The point that yields the most significant result may be taken as the location of the putative QTL.

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Testing genotype-phenotype association in trisomic data. *E. Feingold*¹, *Z. Xu*¹, *K.F. Kerstann*², *S.L. Sherman*². 1) Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA; 2) Dept Human Genetics, Emory University, Atlanta, GA.

A number of congenital disorders are rare in the general population but more common in individuals with specific trisomies; examples include certain heart defects and leukemias in Down syndrome. With the completion of the genome sequence, it is timely to start searching for genetic variants that are associated with these disorders. We present a "trisomic TDT" - a statistical method for testing genotype-phenotype association using trios of trisomic individuals and their parents. Like the conventional TDT, our method conditions on parental genotypes and thus controls for population admixture and stratification. We also discuss methods for case-control association studies using trisomic data.

Sampling bias in the D' statistic and a bootstrap correction. *K.N. Conneely, G.R. Abecasis, M. Boehnke.* Dept Biostat, Univ Michigan, Ann Arbor, MI.

Lewontin's D' is a measure of linkage disequilibrium (LD) that is well suited for LD mapping and is consequently prevalent in the literature despite its unusually pervasive sampling bias. Although D' is asymptotically unbiased, its sampling bias is substantial and persists for large numbers of chromosomes. In contrast, other measures of LD are subject to smaller sampling biases that decay rapidly with increasing sample size. Nevertheless, D' has desirable properties not shared by the other measures of LD. Given some general assumptions, D' in large populations is independent of allele frequencies and is directly related to the recombination fraction and the number of generations since mutation. The advantages and the widespread use of D' suggest that it is a valuable tool despite its bias and that efforts to mitigate its bias are warranted. Through simulation, we examine the statistical properties of D' for biallelic marker pairs with the dual goals of documenting and reducing the sampling bias. We find that the bias varies with the level of LD and with marker allele frequencies. The bias is most pervasive when $D' \approx 0$ and is virtually non-existent when $D' \approx 1$. Estimates of D' are generally biased away from zero, so in the presence of positive LD they tend to be biased upward. The bias can be as great as 10% for samples of fewer than 100 chromosomes and 5% for samples of 500 chromosomes, and is even more severe in LD studies that use the absolute value of D' . When $D' = 0$, the expected value of estimated $|D'|$ ranges from 0.1 to 0.8 in a sample of 100 chromosomes. This type of bias will contaminate comparisons of LD across genomic regions and between samples of varying sizes, leading to the illusion that LD extends much further in smaller samples. To address this problem we propose a bootstrap estimator of D' which outperforms alternative estimators. Our simulations show that bootstrapping D' reduces its bias without increasing its mean squared error. Accordingly, bootstrapping should increase the validity of comparisons of LD across different populations and regions of the genome while fully utilizing the available information. To illustrate this, we use our methods to compare LD maps across populations with different sample sizes.

Simulation of LD block-structured SNP haplotype data and its use for the analysis of case-control data by supervised learning methods. *M. Nothnagel*. Dept. of Bioinformatics, Max Delbrück Center, Berlin, Germany.

Recent findings indicate that haplotypes of single nucleotide polymorphisms (SNPs) exhibit a block structure characterized by high degrees of linkage disequilibrium (LD) between SNPs from the same block and low degrees of LD between SNPs that are from different blocks. Also, only a small fraction of all possible block haplotypes occurs with notable frequencies. To find optimal approaches for analyzing these data, it is desirable to study various methods in well-defined settings. A software program, SNaP, was developed to simulate SNP haplotype data for an unlimited number of individuals, either using random sampling or sampling case-control data. Each individual can be assigned either an affection status or a quantitative trait, conditional on the genotype at one or more loci. Various parameters including penetrances, allele frequencies, and phenotypic effects can be specified. The haplotype is composed of LD blocks, each being defined by pre-set haplotypes and their frequencies. Additionally, SNaP allows for the incorporation of genotyping errors and the removal of causal SNPs from the sequence.

SNaP was used to carry out a simulation study where supervised learning methods (SLMs) were investigated for the analysis of SNP genotype data in a case-control design. One of SLMs' aims is to find the set of SNPs which can best discriminate between cases and controls, thus hopefully pinpoint differences that are causal of the disease status. For each model in a grid spanned by parameters like sample sizes, allele frequencies, and locus interaction and disease definition, data sets were repeatedly simulated using SNaP and various standard SLMs were applied to them. First results for single and two-locus models support the usefulness of classification trees whereas linear and quadratic discriminant analysis seem to be inappropriate. An essential requirement for reliable findings is sample sizes of at least 100 individuals per group. The better methods perform well in all models for penetrance values above 0.8 or susceptibility allele frequencies below 0.25. In general, recessive and multiplicative or heterogeneity models constitute advantageous settings.

Power and significance when screening SNPs in large case-control studies. *K.L. Lunetta, K.G. Ardlie.* Genomics Collaborative Inc, Cambridge, MA.

Under the scenario of candidate gene association studies (case-control, pharmacogenetic, etc.) that look at hundreds of SNPs in large numbers of genes, one preliminary analysis goal is to identify SNPs and more generally genes for follow-up analyses or further genotyping. In this context, one strategy is to consider any gene with at least one positively associated SNP a potential hit worth further study. The choice of test and significance level for declaring a hit depends on the goals of the study. Several strategies for screening large numbers of SNPs for individual associations in large case-control studies are possible. One is to use a 2df test that does not assume a genetic model, or alternatively, a 1df test assuming a specific model. Another possibility is to test more than one possible genetic model for an arbitrary allele (e.g., dominant, additive, and recessive) and compare the maximum statistic to the critical value. This latter strategy must overcome the problems inherent with multiple testing and correlated tests; the critical values used must be increased in order to avoid excessive false positives. We have computed the correlations among dominant, additive, and recessive codings of the Armitage trend test under the null hypothesis, and determined corrected critical values for the maximum Armitage trend test statistic (maximum trend test, MTT). We compare the power of Fishers exact (2df) test and the MTT and find that they are nearly identical under most situations, and that the MTT is marginally more powerful in some low-power situations. We next extend our study to multiple correlated SNPs in a gene. In the genome-wide candidate search context, where multiple SNPs are tested in each candidate gene, we may want to declare a gene a hit worth further study if any single SNP has a positive association, or if there is a positive haplotype association within the gene. We use simulation to determine reasonable criteria for acceptable levels of false positive and false negative hits.

A genotype-based association test for general pedigrees: The geno-PDT. *E.R. Martin, M.P. Bass, E.R. Hauser.*
Center for Human Genetics, Duke University Medical Center, Durham, NC.

Many family-based tests of linkage disequilibrium (LD), such as the pedigree disequilibrium test (PDT), are based on counts of alleles and are most powerful when the disease alleles at a locus act additively on disease risk. When alleles do not act additively, for example when the disease allele is dominant or recessive, tests based on genotype counts may be more powerful. We propose a test for association of genotypes with disease, the geno-PDT. The test is a simple modification of the PDT, and like the PDT, it is valid even in stratified populations. The geno-PDT is applicable to nuclear family or extended pedigree data. The statistic can be used to test any specific genotype or a global test can be used to test all genotypes simultaneously. The null hypothesis is that there is either no allelic association or no linkage between the disease and marker loci, thus the test will have power only when both association and linkage (i.e., LD) are present. The PDT and geno-PDT both test the same null hypothesis but can have different powers depending on the genetic model. We have conducted simulations to examine the false-positive rate and power for the geno-PDT and to compare the powers of the PDT and geno-PDT under different disease models. Genotypes for biallelic disease and marker loci were simulated in nuclear families allowing for variable levels of LD. Simulations show that individual tests and the global geno-PDT have the correct false-positive rates. Power for the global geno-PDT was somewhat higher than power for the PDT under dominant and recessive models, while the PDT tended to be more powerful for the additive model. Applying the geno-PDT to individual genotypes can lead to even more significant results when the model is dominant or recessive and can give important clues as to association at the genotypic level. The geno-PDT will be a useful tool for testing for LD in family data at SNPs. Furthermore, the extension of the geno-PDT to genotypes at multiple SNPs will allow one to test for multilocus effects without the ambiguities associated with haplotype analysis, and using markers in different genes will allow testing of gene-gene interaction.

Assuming independent male-female (m-f) recombination fraction (RF) can reveal imprinting. *D.A. Greenberg^{1,2}, B. Feenstra¹, S.E. Hodge^{1,2}*. 1) Div Stat Genetics, Columbia Univ.; 2) Div Clin Genet Epi, NY Psych Institute, NY NY.

In a linkage analysis of Juvenile Myoclonic Epilepsy [Greenberg et al AJHG 66:508], we noted a statistically significant difference between maximum lod scores (Z_{max}) calculated assuming equal m-f RF (the "restricted" Z_{max}) and Z_{max} allowing independent m-f RF ("global" Z_{max}). We also observed a surprising pattern in the distribution of independent m-f lod scores suggestive of heterogeneity. Because this occurred in a genomic region not known to have sex-based RF differences, we suggested this difference could arise from imprinting [Smalley, AJHG 52:210]. Or the observed difference might arise from ascertainment bias if study subjects are more likely to cooperate when family history exists on the paternal (or maternal) side. Here we answer three questions: 1) Can linkage analysis using differing m-f RFs reveal the existence of imprinting? 2) Can ascertainment bias lead to similar results? 3) How does heterogeneity affect the pattern of lod scores in imprinting? We simulated family linkage data with and without "imprinting" (defined as penetrance dependent on sex of the transmitting parent), but with no difference between m-f RFs. We then determined the difference between the global and restricted Z_{max} values. We found: 1) Restricted vs global differences were statistically significant in realistic samples. For ex., datasets of 20 nuclear families generated with complete imprinting yielded differences of approx. 1.5-2.5 lod score units - statistically significant at least at the $p < .05$ level after correcting for multiple tests. 2) Ascertainment bias based on parental sex does not lead to any differences in global vs restricted Z_{max} and thus does not mimic imprinting, no matter how great the bias. 3) When there is heterogeneity plus imprinting, using independent m-f RF can greatly increase the evidence for linkage as well as yield evidence of imprinting. [Support: NIH DK31775, NS27941, MH48858].

Allele-sharing linkage analysis on subsets of families stratified based on an associated allele. C. Li, L.J. Scott, M. Boehnke. Dept Biostatistics, Univ Michigan, Ann Arbor, MI.

To fine map genes we often test for disease-marker association in regions with previous evidence for linkage. Given an associated allele, we next ask if this allele, or one in linkage disequilibrium with it, could account for part of the observed linkage signal. We propose to address this question by determining if families selected based on an associated allele show stronger evidence of linkage. One possible subset is the set of families in which any affected member carries the allele; a second is the set of families in which all affected members are homozygous for the allele (Horikawa et al. 2000). Unfortunately, in the region of the marker, standard linkage analysis on the first subset is biased *against* linkage, reducing or even removing possible linkage signals, while that on the second subset is biased *for* linkage, artificially generating or amplifying linkage signals. Horikawa et al. proposed to correct this bias by simulations.

For affected sibships, one unbiased solution is to select families based on the genotype of one random sib. However, this subsetting scheme generates different subsets of families depending on the sib chosen, and introduces substantial variability. To make full use of the sibship information, we consider all possible resulting subsets, and observe that each family is selected into a fraction of the subsets, with the fraction being determined based on the genotypes of all affected sibs. This introduces a family weight variable and an unbiased weighted subsetting scheme. We define its corresponding NPL (Kruglyak et al. 1996) and LOD scores (Kong and Cox 1997).

To assess the significance of a result given evidence of linkage, we introduce a permutation test, in which we essentially test for association of a family weight variable with excess allele IBD sharing. Simulations show that this is a powerful test for moderate sample sizes. For example, for 500 ASPs and $I_s=1.1$ and significance level .01, if the disease allele frequency $p=.2$, the powers for additive, dominant, and recessive models are 93%, 94%, and 100%; if $p=.5$, the powers are 83%, 55%, and 98%.

Effects of prior screening for known mutations on power of sib-pair linkage studies. *R.A. Kerber¹, C.I. Amos², D.C. Thomas³, D.M. Finkelstein⁴, B.Y. Yeap⁴.* 1) Huntsman Cancer Institute, University of Utah, Salt Lake City, UT; 2) Department of Epidemiology, Box 189, 1515 Holcombe Blvd, M.D. Anderson Cancer Center, Houston, TX 77030; 3) Department of Preventive Medicine, University of Southern California, 1540 Alcazar Street, CHP-220, Los Angeles, CA 90089-9011, USA; 4) MGH Biostatistics, 50 Staniford St, Ste 560, Boston, MA 02114-2521.

If susceptibility to a disease can be caused by mutations of any of several unlinked genes, linkage analysis becomes more difficult because only a subset of families under study will be contributing to the linkage scores at any given locus, resulting in a loss of power. In designing a Cancer Genetics Network-Cooperative Family Registries for Colorectal Cancer Studies sib-pair study of colon cancer, we wondered whether the power of the study would be improved by excluding families segregating an already-identified predisposing mutation prior to genotyping marker data. To study this question, we performed a variety of simulations of a disease-susceptibility syndrome similar in its general characteristics to colorectal cancer (population rate = 2.2%), with two unlinked moderate-penetrance (27% risk to carriers) susceptibility syndromes present in the population with varying allele frequencies (.001 .005). We simulated a set of 500 families selected for analysis, each with two affected and two unaffected sibs sampled. We compared the power of a test for linkage at a recombination fraction of 0.05 from one disease locus employing all available families with a test for linkage after all families with a mutation at the other locus had been eliminated. We compared a test of mean allele sharing identical-by-descent (IBD) among affecteds only to a test of the mean IBD difference between affected pairs and discordant pairs of sibs. Our results show little difference in power between the designs if the proportion of disease attributable to the linked locus is larger than the proportion of disease attributable to the unlinked locus. However, if the linked locus accounts for a smaller proportion of the disease risk than the unlinked locus, experimentally eliminating families with mutations in the unlinked gene can result in increased power.

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Dissecting The Correlation Structure Of A Bivariate Phenotype For QTL Mapping. *S. Ghosh, T. Reich.* Dept Psychiatry, Washington Univ Sch Medicine, St Louis, MO.

One of the current challenges of genetic epidemiology is to unravel the genetic architecture of complex traits. Heritable quantitative characters, possibly correlated, generally underlie complex traits. Studies have revealed that a bivariate linkage analysis for two correlated traits is often more powerful than separate univariate analyses. However, a high correlation between two quantitative traits need not necessarily imply a common QTL controlling both the traits, but may be due to common environmental factors. The aim of this study is to develop statistical methods to extract the genetic contribution to the total correlation between the components of a bivariate phenotype. Using data on bivariate phenotypes and marker genotypes for sib-pairs, we derive an expression for the conditional cross-sib trait correlations (trait 1 of sib 1 - trait 2 of sib 2 and conversely) given the i.b.d. sharing at a marker locus. We show that, similar to the traditional Haseman-Elston regression (1972), the conditional correlation is a linear function of the marker i.b.d. sharing under low degrees of dominance at the traits. A test for linkage with a common QTL can be performed based on the coefficient associated with the marker i.b.d. sharing, which is zero under the null hypothesis of no linkage and positive in the presence of linkage. Monte-Carlo simulations are included to assess the performance of the proposed procedure. We also present an application of our method to two correlated alcohol related phenotypes in the COGA data. Univariate studies have obtained linkage in the ADH cluster on Chromosome 4 with maximum number of drinks in a 24 hour period as well as number of externalizing symptoms. Our correlation-based test yields a p-value < 0.001 with the ADH3 marker, suggesting a potential candidate gene.

Magnitude of Lod Score Differences from Unequal Male-Female Recombination Fractions. *B. Feenstra, S.E. Hodge, D.A. Greenberg.* Div. of Stat. Genet., Columbia University, New York, NY, and New York State Psychiatric Institute, New York, NY.

It is well-known that human recombination fraction (RF) can differ between males (m) and females (f). However, only recently have investigators begun to allow for or test this difference in linkage analyses. We quantified the effect of differential m-f RFs on linkage analysis for fully informative gametes (FIGs). (FIG = a gamete that is unambiguously either recombinant or nonrecombinant.) We calculated expected lod scores (ELODs) under two different conditions: 1) allowing sex-specific recombination fraction parameters (q_m, q_f) and 2) assuming $q_m = q_f$. We then examined the DELOD, defined as the difference between ELODs maximized as a function of (q_m, q_f) under condition 1) vs. 2). The magnitude of the DELOD depends on the true RFs (r_m and r_f), on sample size, and on the proportion (p) of paternally-derived (or $(1 - p)$ of maternally-derived) FIGs. We calculated minimum sample sizes required to achieve DELOD ³ 0.83, corresponding to a statistically significant c^2 at the 5% level.

(r_m, r_f)	(0.05,0.2)	(0.01,0.2)	(0.01,0.3)	(0.01,0.4)	(0.01,0.5)
# FIGs required	70	33	20	14	10

We also found that for any given sample size, DELOD is maximized for p between 0.36 and 0.64, depending on (r_m, r_f). Finally, we examined DELOD for sib pairs that are fully informative except for having phase-unknown parents, and found similar results. We note that other phenomena, such as imprinting, may also lead to lod score differences between analysis under conditions 1) vs. 2). This study shows the magnitude of differences that can be expected from differential m-f RF.

Fast evaluation of parametric models in pedigree analysis. *G.R. Abecasis.* Center for Statistical Genetics, Department of Biostatistics, University of Michigan, Ann Arbor.

Parametric analyses allow explicit modeling of phenotype and genotype data. Traditionally, parametric models are specified for the trait locus and provide powerful linkage tests when the trait model is specified accurately. More recently, parametric analyses have become important in the modeling and detection of genotype errors and in the area of relationship inference. Although these applications are important in the mapping of complex traits, the evaluation of parametric models is very computationally demanding in many settings. For example, likelihood calculations that incorporate trait models with multiple alleles or allow for genotype error most often rely on approximate Monte-Carlo methods.

We describe an efficient computational approach for evaluating parametric likelihoods for individual inheritance vectors. Our approach has the following features: 1) binary trees are used to represent gene flow in pedigrees, allowing for gradual updates of likelihoods and reducing redundant calculations; 2) efficient orderings are used for evaluation of nested sums within each inheritance vector; 3) for suitable genotype error models, allele set recoding is applied to individual founder allele sets to simplify calculations and 4) optionally, greedy evaluation is used to skip unlikely inheritance vectors and founder allele sets implying large numbers of errors. Our model allows both for trait loci with multiple alleles and explicit modeling of genotyping error.

We show that our approach provides near instantaneous likelihood calculations for small pedigrees (such as sib-pairs) but can also handle larger pedigrees for either SNP or microsatellite data. Without explicit error modeling, undetected genotyping error greatly reduces power for linkage analyses. In the context of affected sib-pair linkage analysis, we use simulation to compare the efficacy of a) error detection and removal and b) explicit modeling of genotype error in recovering lost linkage information. We expect that results of this comparison will help investigators devise strategies for managing genotyping error.

Detecting low-quality markers using map expanders. *C.T. Ekstrøm*^{1,2}. 1) Department of Biostatistics, University of Copenhagen, Denmark; 2) Steno Diabetes Center, Denmark.

Genetic marker data play a crucial role in gene mapping and genotyping errors can therefore have substantial influence on the power to detect and the precision to locate disease loci. Statistical methods can be used to identify individuals, markers or pedigrees with high likelihood of containing genotyping errors. Putative erroneous genotypes can then be rechecked and either verified or corrected to reduce the loss of power introduced by the errors.

We present a method to detect genetic markers with high genotyping error rate. Genotyping errors are likely to appear as double recombinations, which will expand the genetic map around the marker. Markers flagged as map expanders (i.e., having an excessive number of double recombinations) can then be reread or regenotyped or a replacement marker of higher quality can be used instead. The method can be applied to any type of pedigree.

A simulation study of nuclear pedigrees and sib pairs shows that the proposed method generally has high power to identify map expanders when the set of markers is reasonably dense (e.g., an average marker distance of 5 cM) - even when the genotyping error rate is low (<2%). Not surprisingly, the power to detect map expanders increases with marker heterozygosity and genotyping error rate and is reduced with increasing inter-marker distance.

Linkage with multivariate summary measures of correlated phenotypes. *D.L. Koller, S.L. Hui, P.M. Conneally, C.C. Johnston, M.J. Econs, T. Foroud, M. Peacock.* Indiana Univ. Sch. of Medicine, Indianapolis, IN.

In the study of complex genetic disease, multivariate summary measures are often used to analyze the genetic contribution to related phenotypes. Inferences are often made from these analyses as to pleiotropic effects of the loci detected. We present the results of a linkage study in which a multivariate summary measure and a true composite phenotype were available for comparison. We ascertained a sample of 774 premenopausal sister pairs. Bone mineral density (BMD) at the hip was obtained for all sisters by DXA at femoral neck, trochanter, and Ward's triangle, as well as for total hip, which includes the three specific sites. A 10 cM genome scan was performed by CIDR. Principal component scores for BMD at the three hip sites were obtained using SAS. LOD scores were calculated with Mapmaker/SIBS. The three specific hip BMD measures and total hip BMD were highly correlated within individuals in our sample ($r=0.81-0.91$). Maximum LOD scores for chromosomes 14 and 15 for the 3 specific hip BMD measures, as well as for total hip and the first principal component (which explained 90% of total variation), are shown below. We conclude from these data that site-specific genetic effects may be detectable even among the highly-correlated phenotypes most amenable to multivariate analysis, and that genetic information may be lost in summary measures such as principal components. These data also show a substantial difference in linkage results using a true composite measure and a multivariate summary measure, implying that composite phenotypes may include non-redundant genetic information in addition to that contained in site-specific and summary measures.

Chromosome	Trochanter	Ward's tri.	Femoral neck	1st pr. comp.	Total hip
14	3.47	2.35	1.83	2.85	1.36
15	0.88	2.87	4.25	2.67	1.31

Linkage analysis of Alzheimer's Disease (AD) with latent class models. *R.J. Neuman¹, A. Myers¹, L. Sun¹, J. Williams², J.P. Rice¹, N.L. Saccone¹, A. Goate¹.* 1) Dept Psychiatry, Wash Univ, St. Louis, MO; 2) U. Wales, Cardiff U.K.

Although traditional allele-sharing (IBD) linkage methods play an important role in looking for disease loci, they have several limitations: eg., searching for only 1 trait locus at a time, or not utilizing unaffected individuals thus losing what may be important information. We applied latent class analysis (LCA) to IBD data in a late-onset AD dataset containing 429 ASP and 247 discordant pairs (DSP). LCA can be thought of as a parametric clustering method and, as such, does not have the restrictions of traditional linkage methods noted above. A recent ASP genome scan of these data [Myers et al., 2002] found 10 regions with LOD scores greater than 1. Their maximum MLS, 3.5, occurred at 82.2 cM on chr10. Our LC reanalysis of these data first analyzed markers on each chr separately. The strongest signal was on chr5 in a 9-cluster solution, the solution with the minimum Bayesian Information Criterion. The number of ASPs was greater than expected by chance ($p=0.005$) in a class in which over 97% of pairs shared IBD=2 at markers. This was in the same vicinity of chr5 where Myers et al. reported a LOD>1. Our next strongest signal was on chr10, again in a 9-cluster solution. A high proportion of ASPs ($p=0.07$) were assigned to a cluster in which over 90% shared IBD=2 in the vicinity of D10S1227 (75cM). To detect epistasis arising from non-syntenic regions, markers were analyzed jointly from chr5, 10, and 13 (where no signal was detected by Myers et al., nor by our LCA of chr13 markers). A cluster of sib pairs with an excess of ASPs and excess sharing at markers on chr5 and 10 in the regions detected by Myers et al. was singled out: cluster 8 contained 87.5% ASP versus 63.4% in the total sample ($p=0.002$). For comparison, over 90% of pairs shared at markers on chr13, but only 50% were ASPs, indicating no linkage evidence. AD presents a challenge for genetic analysis because of its uncertain etiology, inconclusive diagnosis before death, and other risk factors. Nevertheless, LCA was able to replicate some of the regions of interest found by Myers et al., and to detect a possible interaction among multiple susceptibility loci.

Evaluating results from genome-wide linkage scans of complex discrete traits by locus counting. *S. Wiltshire*^{1,2}, *M.I. McCarthy*¹, *L.R. Cardon*². 1) Imperial College Genetics and Genomics Research Institute, Imperial College, London, United Kingdom; 2) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom.

Evaluating results from primary (~10cM) genome-wide linkage scans of complex human traits remains an area of considerable debate. In addition to measures of statistical significance, a complementary means of evaluation is possible, based on a comparison of the numbers of Independent Regions showing evidence for Linkage (IRLs) observed in a genome scan, at various LOD score thresholds, with those expected by chance. We have explored by simulation the characteristics of this locus counting method over a range of experimental conditions typically encountered during genome scans of discrete traits, using GENEHUNTER PLUS and treating loci as independent if their maxima are ³40cM apart. Under the null hypothesis of no linkage, factors impacting on data informativeness markedly influence the null IRL distribution. For instance, evidence for linkage with LOD=2.20 is expected <0.3 times by chance per primary scan, compared with once predicted under the assumption of complete information extraction. Our simulations suggest that in a typical primary scan we expect 8-11 IRLs with LOD³0.59 and 2-3 with LOD³1.18 by chance, depending on the precise data characteristics; IRLs with LOD³ are rare. Furthermore, we expect one region by chance with LOD=1.5-1.7. We have applied this method to the Warren 2 genome scan for type 2 diabetes susceptibility. We observe significantly more regions showing evidence for linkage with LOD scores of 1-2.5 in the primary scan than expected by chance (for example, 7 regions observed at LOD³1.18 vs 2 expected), even though no region achieved empirical genome-wide significance. Locus counting may be useful in assessing results from complex trait genome scans in general, especially from scans that fail to generate evidence for linkage reaching genome-wide significance. By accounting for the effects of reduced data informativeness on the expected numbers of IRLs, a more meaningful and less conservative evaluation of results from such studies is possible.

Comparison of methods for QTL mapping with selected sibships. *K. T. Cuenco*¹, *J. Szatkiewicz*², *E. Feingold*¹. 1) Dept of Human Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Dept of Biostatistics, University of Pittsburgh, Pittsburgh, PA.

In the last few years, at least a dozen new methods for mapping quantitative trait loci (QTLs) using sibships have been published. Most of the papers describing the new methods have only considered population samples from normally-distributed traits. It is not at all clear which methods are most appropriate for selected samples, which are arguably more common than population samples in human genetics. We have written software to implement essentially all of the new methods for sib pairs, and have made both theoretical and analytical comparisons of their performance on samples that are selected on the basis of one sib with a high (or low) trait value. While most of the new statistics are asymptotically equivalent for population samples, there are important power differences for selected samples.

New statistical methods for QTL mapping with discordant sibling pairs. *J. Szatkiewicz*¹, *K. T. Cuenco*², *E. Feingold*². 1) Dept of Biostatistics, University of Pittsburgh, Pittsburgh, PA; 2) Dept of Human Genetics, University of Pittsburgh, Pittsburgh, PA.

Discordant sibling pairs can potentially be very powerful for mapping quantitative trait loci (QTLs). Statistical analysis for discordant sib pair studies has traditionally been based on the identity-by-descent (IBD) sharing statistic, though Forrest and Feingold (2000) also proposed a "composite" statistic that combines the IBD sharing statistic with an additional statistic such as Haseman-Elston. In the last few years, however, a number of new statistics for QTL mapping with sibling pairs have been published, and some of these are applicable to discordant pairs. We have reviewed the new statistics and compared their power for mapping using discordant pairs. We have also compared the power of the newer methods to that of the IBD sharing statistic and the composite statistic. We considered both extreme discordant pairs (one sib in the top 10% of the trait distribution and one in the bottom 10%) and moderately discordant pairs (one in the top 35% and one in the bottom 35%).

Power of a simplified multivariate test for genetic linkage. *O. Gorlova*¹, *C. Amos*¹, *D. Zhu*¹, *W. Wang*¹, *S. Turner*², *E. Boerwinkle*³. 1) Dept Epidemiology, Univ Texas MD Anderson CA Ctr, Houston, TX; 2) Division of Hypertension, Department of Internal Medicine, Mayo Clinic, Rochester, MN; 3) Human Genetics Center and Institute of Molecular Medicine, The University of Texas Health Science Center, Houston, TX.

We compare the power of the multivariate Haseman-Elston (MHE) test proposed earlier by Amos et al. (*Am J Hum Genet* 1990, 47:247-254) and a computationally rapid new version of the multivariate Haseman-Elston test (NMHE) (Elston et al., *Genet Epidemiol* 2000, 19:1-17). We show that the power of NMHE was, for different simulation setups, identical or higher than that of MHE. In the bivariate case, the power of the NMHE method was somewhat less than that of the computationally intensive maximum likelihood variance components method (Amos et al, *Hum Hered* 2001, 51:133-144). We present comparisons of the empirical distributions of the NMHE test to its limiting distributions for a range of numbers of traits. The distribution of NMHE test appeared to conform satisfactorily to its limiting asymptotic distribution in large samples. Otherwise, the test proposed by Elston et al. (2000) is non-conservative. The use of empirical critical values is therefore recommended for limited sample sizes (less than several hundred families). We also present the results of a linkage analysis performed by the NMHE method on a set of 4 body size-related traits. The method identified meaningful combinations of traits that showed significant linkage on chromosome 2 and suggestive linkages on chromosomes 16 and 17.

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Finding causal variants of complex diseases via haplotypes. *S. Bacanu*. Dept Psychiatry, 3811 O'Hara Street, E-1221, University of Pittsburgh, Pittsburgh, PA 15213.

The analysis of genetic polymorphisms as haplotypes is thought to be useful to detect causal polymorphisms affecting liability to complex disease. One of the methods transferred to statistical genetics from the data-mining field is the tree classifier. Tree-like classifiers have many good properties but, by themselves, they cannot take multilocus information into account. We propose a method that combines the desirable attributes of tree classifiers with the inherent multilocus information available in haplotypes. Our tree-like method aggregates the haplotypes based on their effect on the trait. It sequentially adds the haplotype with the highest effect on the trait that improves the cross validated log-likelihood significantly. The power and accuracy of the proposed method are assessed by use of simulated data. The performance of more traditional methods is also assessed on the same data for the purpose of comparison. We find the proposed method is excellent at detecting haplotypes bearing variants increasing liability and usually has at least as much power as other, commonly used methods.

Extensions of multipoint methods for localizing susceptibility genes. *D.V. Glidden¹, K.-Y. Liang², Y.-F. Chiu³, A.E. Pulver⁴.* 1) Epidemiology and Biostatistics, Univ. of Calif., San Francisco, San Francisco, CA; 2) Department of Biostatistics, Johns Hopkins Univ., Baltimore, MD; 3) Department of Biostatistics, Univ of N. Carolina, Chapel Hill, NC; 4) Department of Medicine, Johns Hopkins Univ., Baltimore, MD.

Recently, Liang et al. (2000) proposed a general multipoint method for estimating the chromosomal position of a putative susceptibility locus. Their technique is computationally simple and does not require specification of penetrance or a mode of inheritance. This paper considers various extensions including exploration and inference for covariates which may reflect etiologic heterogeneity. Here, we draw particular attention to incorporating age of onset information. The method can be extended, in a similar spirit, to include affected/unaffected sibling and general affected relative pairs. Through simulation studies and a data example, we demonstrate that these extensions allow for robust, precise localization of susceptibility genes for complex diseases.

Unraveling complex traits through the genetics of gene expression. *S.A. Monks^{1,2}, E.E. Schadt¹*. 1) Informatics, Rosetta Inpharmatics, Kirkland, WA; 2) Department of Biostatistics, University of Washington, Seattle, WA.

In 1980 Botstein et al. proposed that sequence differences be treated as markers in order to map genes involved in inherited traits. Since that time, the number of genes mapped to positions in the human genome has grown exponentially. Mapping these genes to inherited traits has been extremely successful for simple Mendelian diseases; however, finding such genes for diseases, and their associated risk traits, that are of public health interest has proven difficult. Reasons for this difficulty include disease heterogeneity (disease sub-types with some or no overlapping genetic causes), misclassification (from using discrete classifications of disease from thresholds and combinations of thresholds), and cumulative environmental influences. With the advent of technology to measure changes in gene expression, i.e., changes in mRNA transcript abundance, it should be possible to unravel some of the complexity existing for these common diseases. We will show that studying the genetic component of mRNA expression is possible through the use of a sample of CEPH (Centre d'Etude du Polymorphisme Humain) families. Discussion will include assessing differential expression of genes for the population, determining whether variation in expression is under genetic control and establishing loci that control variation in expression. Time permitting, it will be shown that combination of information across a number of genes can be used to establish, support and/or confirm a biological pathway.

Association meets haplotype sharing under allelic heterogeneity. *D. Qian*. Dept of Preventive Medicine, Univ of Southern California, Los Angeles, CA.

Despite the increasing evidence of allelic heterogeneity at loci predisposing to complex disease, most statistical methods have been developed and evaluated under a simplified assumption that complex disease is caused by multiple genes, each having minimal allelic heterogeneity. Allelic heterogeneity refers to multiple ancestral disease alleles at a single locus, and this phenomenon is expected to be the rule rather than the expectation for complex diseases in humans. We generate haplotype data with allelic heterogeneity by a forward population simulation procedure, where distinct ancestral alleles and linkage disequilibrium in population haplotypes can be monitored. When markers do not include the true disease locus, the haplotype sharing correlation (HSC) method has the capacity to map disease genes that are missed by transmission disequilibrium test method under allelic heterogeneity and weak LD. The HSC signals occur near the disease locus and show obvious drops at recombination hot spots, but the peaks may not pinpoint the true disease location. The false positive signals in HSC analyses are rare and weak in data sets we analyzed. For case-parent trios, the HSC significance on disease segment obtained using reconstructed haplotypes is much weaker than those obtained using true simulated haplotypes. On the other hand, when the true disease locus is a marker locus, association method is more powerful than HSC method under low frequency of disease alleles, but the two approaches have similar power under high frequency of disease alleles. These results suggested that haplotype sharing analysis is useful for finding genes under allelic heterogeneity and/or high frequency of disease alleles.

Efficiency and robustness of the set association approach for case-control studies with SNPs. *A. Wille*^{1,2}, *J. Ott*¹. 1) Statistical Genetics, Rockefeller University, New York, NY; 2) IMBIE, Bonn University, Germany.

In complex traits, multiple disease mutations with small marginal effects interact to produce the trait. For this reason, it has been difficult to map susceptibility loci by conventional locus-by-locus methods even with high resolution SNP marker maps. Novel fine mapping strategies are needed that focus on the simultaneous detection of interacting disease mutations and combine information over neighboring loci while handling large numbers of densely spaced markers.

Based on these requirements, a set association approach was proposed recently for case-control association studies with uncorrelated SNPs. In a stepwise selection process, markers are combined in a set whose overall association is evaluated by the sum of the single marker signals.

Here, the efficiency of the approach is examined depending on the underlying disease model, the sample size and the properties of the marker data. It is shown that the set association method performs particularly well when densely spaced markers with a homogeneous inter-marker correlation pattern are used in the study. However, when the correlation structure varies between susceptibility and non-susceptibility regions, the power to detect set association can be greatly reduced. An extension of the approach in which inter-marker linkage disequilibrium is taken into account proves to restore this loss of power.

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A multilocus association test for case control data with improved flexibility of the model assumptions. *D.V. Zaykin, M.G Ehm.* Dept Population Genetics, GlaxoSmithKline, Inc, Res Triangle Park, NC.

Allelic and genotypic chi-square tests are often used when testing for association on unrelated individuals. The extension to multiple loci results in haplotypic and multilocus genotypic tests. Although haplotypic tests assume (and can detect) only additive effects, the majority of biologically realistic genotype-driven models nevertheless marginally induce considerable additive effects of haplotypes. Haplotypic tests can be more powerful in these situations because the number of parameters involved is reduced. When gametic phase is unknown, the commonly used likelihood ratio test (LRT) incorporates an additional assumption of Hardy Weinberg equilibrium (HWE). We construct a new case-control test that involves the same number of parameters as the haplotypic LRT but does not assume HWE. The test can be considered as a genetically motivated way of reducing dimensionality of the data. We derive the asymptotic distribution of the test statistic and compare its properties with the LRT, the multilocus genotypic test, and the allelic tests on individual SNPs under variety of models. We report optimal properties of the new method and improved robustness with respect to the disease model and HWE.

The Importance of Model Validation in Determining the Clinical Utility of Genetic Interactions on Disease Risk.

*C.S. Coffey*¹, *P.R. Hebert*², *M.D. Ritchie*³, *H.M. Krumholz*², *J.M. Gaziano*⁴, *P.M. Ridker*⁴, *J.H. Moore*³. 1) UAB School of Public Hlth Birmingham, AL; 2) Yale Univ. School of Medicine New Haven, CT; 3) Vanderbilt Univ. School of Medicine Nashville, TN; 4) Brigham & Women's Hospital Harvard Medical School Boston, MA.

It is a well-known problem in statistics that models obtained using data-driven methods are prone to increased type 1 errors and, hence should always be examined for validation. Although there is general agreement regarding the importance of model validation, this problem is often ignored in published research possibly due to the fact that correction for overestimation often leads to less significant results. We illustrate the use of model validation for an analysis using data from the Physicians Health Study to examine a possible interaction among the angiotensin converting enzyme (ACE) insertion/deletion and plasminogen activator inhibitor-1 (PAI-1) 4G/5G gene polymorphisms on risk of myocardial infarction. Initially, the Multifactor Dimensionality Reduction Method (Ritchie et al. *AJHG* 69, 2001) found a significant interaction between these polymorphisms on risk of MI when applied to data obtained from 343 matched case-control pairs. However, using ten-fold cross-validation, the prediction error for the final models was only 46%, indicating that the model was only slightly better than that yielded by a coin toss (50% prediction error). After completion of the initial study, an additional 141 case-control pairs became available as a validation sample. The model developed in the initial study did not validate in this independent sample (52% prediction error). Furthermore, the interaction was no longer significant. This example reminds us that the purpose of research is not to obtain 'significant p-values', but rather to uncover relationships that can lead to improved treatments, therapies, or understanding of disease processes. As data-driven analytic methods continue to be developed and used to examine complex genetic interactions, it will become increasingly important to stress model validation in order to ensure that significant effects represent true relationships rather than chance findings.

Analytical methods for factors modifying disease risks in carriers of susceptibility alleles. *D.F. Easton¹, A. Antoniou¹, D.E. Goldgar²*. 1) Cancer Research U.K. Genetic Epidemiology Unit, University of Cambridge, Cambridge, U.K; 2) International Agency for Research on Cancer, Lyon, France.

There is currently great interest in identifying factors, either genetic or environmental, that modify the risk of disease in carriers of known susceptibility genes such as BRCA1 or BRCA2. In order to study large numbers of carriers, most such studies are based on retrospective analysis of affected and unaffected individuals, often ascertained through multiple case families. Analysis of such data is often performed using standard survival analysis techniques (e.g. Cox regression). Although this approach can give valid significance tests, it gives rise to biased relative risk estimates. We propose two alternative types of analysis. The first, a "full" likelihood analysis, is applicable to fixed covariates, e.g. genotypes. It can be conducted with unrelated individuals or with pedigree data, and we have implemented this in the program MENDEL. A full likelihood analysis is, however, not feasible for most lifestyle risk factors, which are time-dependent. As an alternative, we propose a method based on weighting observations so as to mimic a true cohort, and using these weighted observations in a standard survival analysis. Optimal choice of weights, and comparisons between these methods and a standard survival approach, will be discussed. We examine specifically examples from the IBCCS dataset on lifestyle risk factors in BRCA1/2 carriers.

The Maternal-Fetal Genotype Incompatibility Test to Detect Adverse Intrauterine Conditions. *S.L. Minassian*¹, *C.G.S. Palmer*^{2,6}, *J.A. Woodward*^{5,6}, *J.S. Sinsheimer*^{1,3,4}. 1) Departments of Biostatistics; 2) Psychiatry & Biobehavioral Sciences; 3) Human Genetics; 4) Biomathematics; 5) Psychology; 6) Statistics, University of California, Los Angeles.

Current research indicates that maternal-fetal genotype incompatibility may be a risk factor in a variety of diseases. We developed a maternal-fetal genotype incompatibility (MFG) test to examine hypotheses about incompatibility in the possible presence of linkage disequilibrium (LD) at a candidate gene. For a bi-allelic locus, the MFG test uses genotype information to delineate case-parent trios into 15 categories resulting from 6 mating types. This family-based design extends the case-parent log-linear model for trios. The expected number of each type of trio is modeled by a mating type parameter, an incompatibility relative risk parameter, and an LD relative risk parameter. The significance of incompatibility and LD effects can be tested using asymptotic test statistics such as the likelihood ratio test (LRT). The asymptotic chi-square LRT has adequate power with moderate effect size and sample size. As an example, for an incompatibility relative risk of 2.6, an allele frequency of 0.35 for the less common allele, and in the absence of LD effects, 150 trios will yield 88% power to detect the incompatibility effect, and 250 trios yield 98% power. The MFG test also has more power to detect incompatibility effects than the Gamete Competition model (which tests the incompatibility effect directly) or models that can indirectly test for the effect like the Transmission/Disequilibrium Test. In small samples, permutation p-values can be more accurate than asymptotic p-values. Thus, we have developed a permutation test to estimate p-values in small to moderately sized samples. The permutation scheme depends on the hypothesis to be tested. We always permute within trio categories that have the same expected number of counts under the null hypothesis, but different expected number of counts under the alternative hypothesis. We then compare the p-values obtained by the permutation test and asymptotic test. Finally, we give general guidelines for when to use each test.

Multifactor dimensionality reduction is an ideal discriminator of discrete clinical endpoints using multilocus SNP genotypes. *J.H. Moore, L.W. Hahn.* Program in Human Genetics, Vanderbilt Univ, Nashville, TN.

The identification and characterization of genes whose effects are primarily through interactions with other genes and environmental factors remains a statistical and computational challenge in the genetic epidemiology of common, complex multifactorial diseases. We have previously developed a multifactor dimensionality reduction (MDR) approach to identifying gene-gene and gene-environment interactions in case-control and discordant sib-pair study designs. MDR is nonparametric in that no parameters are estimated and is genetic-model free in that no particular genetic model is assumed. Using both simulated and real data, we have demonstrated that MDR has excellent power for identifying high-order gene-gene interactions in the absence of any detectable independent main effects of each gene. Further, we have demonstrated that the power of MDR to identify gene-gene interactions is robust in the presence of genotyping error and phenocopy. In the present study, we outline a mathematical proof that MDR ideally discriminates between discrete clinical endpoints using multilocus SNP genotypes. In this proof, we first define the context of the problem, the MDR decision rule, and the error evaluation on which the decision rule is optimized. We then prove that the MDR decision rule is optimal for the given error evaluation. This proof suggests that no analytical approach will classify discrete clinical endpoints using multilocus genotypes better than MDR. Based on this proof, we propose that MDR should be the "gold standard" with which other methods for identifying gene-gene interactions are compared.

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A novel strategy for selecting optimal subsets of SNPs for the analysis of gene-gene interactions. *B.C. White, L.W. Hahn, J.H. Moore.* Program in Human Genetics, Vanderbilt University, Nashville, TN.

A dense map of single nucleotide polymorphisms (SNPs) is expected to facilitate the identification of disease susceptibility genes. A complicating factor is the reality that the effects of some genes will only be detected when considered in the context of other genes (i.e. epistasis). The implication of this reality for using SNPs to identify disease susceptibility genes is that each SNP must be considered in the context of other SNPs. The evaluation of subsets of SNPs presents an enormous combinatorial problem when the total number of SNPs is large. Even with an accurate haplotype map, powerful computer systems and efficient search algorithms will be needed to facilitate the identification of optimal SNP subsets from an effectively infinite number of possibilities. We have implemented and evaluated a novel parallel search algorithm that is inspired by the problem solving abilities of ant colonies for food foraging. With this ant colony optimization (ACO) approach, each artificial ant initially carries out a random walk through multilocus SNP space. Each set of SNPs selected by an artificial ant is statistically evaluated and the probability of selecting those SNPs is increased or decreased based on how well the set of SNPs distinguishes affected from unaffected individuals (i.e. the statistical fitness). This is analogous to updating a pheromone trail. Many ants are evaluated and the process of updating the virtual pheromone trail is iterated until the artificial ant colony converges on a best SNP subset (i.e. a single pheromone trail to a food source). Using simulated data with 10,000 SNPs, we demonstrate the ACO approach is competitive with other search methods such as parallel genetic algorithms. The simplicity and efficiency of the ACO approach make this an attractive algorithm for the development of SNP subset selection software.

The multivariate Dale model and genetic associations. *K. Van Steen*¹, *G. Molenberghs*¹, *N. Tahri*^{2,3}. 1) Center for Statistics, Limburgs Universitair Centrum, Diepenbeek, Belgium; 2) INSERM U.525, C.H.U. Pitié Salpêtrière, Paris, France; 3) Genset Genomics Research Center, RN7 site snecma, Evry, France.

In a first attempt to investigate the potential merits of the multivariate Dale model (Molenberghs and Lesaffre 1994) when studying genetic associations, we assess the beneficial use of the model in a case-control setting, using unrelated individuals. In the multivariate Dale model joint probabilities are decomposed into main effects (described by marginal probabilities) and interactions (described by cross-ratios of second and higher orders).

Encouraged by promising results in the first stage described above, we then investigate the usefulness of the multivariate Dale model in modeling associations between genes, using family data or related individuals. In particular, we investigate how the model can be used as a means to relate the interplay between subsets of genes to different aspects (symptoms) of a disease.

Because of the extra complexity (elaborate familial inter-relationships), special attention is given to the choice of the estimation procedure. We recommend to adopt a pseudo-likelihood estimation technique (above GEE2) when both multivariate outcomes and (family) clustering are involved.

All research results (pros and cons) will be discussed in practical settings, such as data on late-onset Alzheimer's disease.

Study Design, Family History Measure, Confounding: Which cause discrepancy in effect estimates in Familial Aggregation Studies? R. Zimmerman, H. Ahsan, D. Greenberg. Department of Epidemiology and Division of Statistical Genetics, Columbia University, New York, NY.

Case control (CC) and reconstructed cohort (RC) study designs of familial aggregation of disease have been shown to result in discrepant effect estimates even when the exact same data set is being assessed (Khoury and Flanders, 1995). Researchers demonstrated that the effect estimate resulting from a CC design becomes more discrepant from the effect estimate derived from the RC with increasing disease prevalence and increasing rate ratio derived from the RC. They concluded that the discrepancy results from bias in the CC study. They also concluded that confounding by family size and age in the CC study may account for some of the discrepancy between effect estimates derived from each study. We do not agree with these findings. We provide a rationale for why we disagree and demonstrate the causes for discrepant effect estimates derived from the CC and RC. We also explain why neither number nor age of relatives *naturally* confounds the family history disease association in the CC study. Using simulated populations (Greenberg, MacCluer, et al. 1999) as the sampling frame for three epidemiologic familial aggregation study designs, Cohort, CC and RC, we compare effect estimates derived from identical populations and find that our hypotheses are correct. The discrepancy between effect estimates is due to the variation in family history measures (Hopper, 1996) not the study design. By distinguishing between family member(s) that define exposure and those that define disease, we also demonstrate that family size and number of relatives defining the exposure measure do not confound the family history - disease association. We conclude that the RC does not pose any benefit over the CC design in studies of familial aggregation and decisions on which to use depends on the particular specifications of the familial aggregation study.

Microsatellite Variation: Effects of Natural Selection, Population Structure, and Demographic Changes of Population Size. *H. Xu*¹, *Y.-X. Fu*¹, *M. Kimmel*², *A. Renwick*², *R. Chakraborty*³. 1) Human Genetics Ctr, Univ. Texas School of Public Health, Houston, TX; 2) Statistics Department, Rice University, Houston, TX; 3) Center for Genome Information, Department of Environmental Health, Univ. Cincinnati, Cincinnati, OH.

Statistical properties of all measures of genetic variation critically depend upon the composite parameter, $q = 4N_e m$, defined by the product of effective population size (N_e) and the rate of mutation (m) of the locus. Consequently, the differences of estimates of q based on alternative summary measures of variation have been used to develop test statistics to detect effects of natural selection, presence of population substructure, and signatures of past demographic history of populations. In this investigation, we show that these currently used test procedures cannot be uncritically used to analyze data on microsatellite loci. Based on analytical and simulation studies of coalescent theory, we show that for microsatellite loci evolving via a generalized stepwise mutation model, the number of segregating alleles (k) in a sample is not a sufficient statistic for the parameter q . Consequently, the relationship of expected homozygosity (F) and the number of segregating alleles (k) in a sample is different as q changes. Furthermore, the presence of population substructure differentially impacts the relationship of expectations of F and k , depending upon the value of q . For example, for small q (say, $q < 1$), substructuring generates an excess of F for a given k , while for large q (say, $q > 10$), the relationship is reversed (i.e., a deficiency of F is observed, like the effects of overdominant selection). As a consequence, the assertion that population substructure mimics the effect of overdominant selection appears to be true only for microsatellite loci with a comparatively higher mutation rate and/or in populations of large effective size. In contrast, exponential growth of populations always generates an excess of F for a given k compared with a population of constant size, and thus, it confounds the effect of directional selection on microsatellite variation irrespective of population size and the mutation rate.

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Bayesian Classification for Genome-Wide Control Using Multiple Unlinked Haplotype Blocks. *T. Niu*¹, *Z.S. Qin*², *J.S. Liu*². 1) Prog Population Genetics, Harvard Sch Public Health, Boston, MA; 2) Dept of Statistics, Harvard Univ, Cambridge, MA.

Case-control study is a power approach to identify susceptibility genes for complex traits. However, hidden population substructure can lead to spurious associations between the genetic marker and the disease. The recent advancement in biotechnology has allowed unprecedented speed and throughput in genotyping a voluminous number of SNPs. Thus, a standard set of unlinked SNPs that uniquely capture all possible common haplotype blocks in the human genome can be selected as genome-wide controls. We developed a Bayesian classification approach to utilize the unlinked haplotype data to assign subpopulation membership for each individual. We show that our method is robust and can reveal the underlying cryptic relations to correct for the population substructure in case-control analyses, which can effectively minimize the false-positive rates in discovering gene-disease association. This method can also be used for clustering haplotypes that will increase the power for linkage disequilibrium mapping.

Likelihood measures and methodology for distinguishing single vs. multiple DNAs using STRs in samples for epidemiologic studies. *S. Shrestha*^{1,2}, *S.A. Strathdee*¹, *T.H. Beaty*¹, *M.W. Smith*². 1) Genetic Epidemiology, Johns Hopkins University, Bloomberg School of Public Health, Baltimore, MD 21205; 2) Basic Research Program, SAIC-Frederick, National Cancer Institute, Frederick, MD 21702.

We developed a new likelihood measure of mixture discrimination to distinguish single from multiple genomes in biological samples. This likelihood approach uses short tandem repeats (STRs) as biomarkers and combines theoretical approaches in genetics, forensic science, epidemiology and bio-informatics. Exact probability estimates account for all possible combinations of STR alleles providing a uniquely informative approach to identifying mixtures. Theoretical frameworks have been developed for autosomal markers, plus X- and Ylinked STRs in all male/male, male/female and female/female mixtures. These analyses indicate that the cost of genetically determining the presence of male and female genetic material in an unknown sample outweighs the benefits of simpler interpretation of X and Y chromosome STRs. Thus, the laboratory methods focus on four autosomal STRs with heterozygosity >75% which provide a 99% probability of detecting mixtures of DNA. Pentanucleotide repeats are the STRs of choice since they have minimal slippage during PCR, resulting in simpler patterns of amplification and minimizing false positive alleles. Genetically determined mixture information can be used as surrogates for physical or behavioral characteristics in epidemiologic studies to examine molecular and infectious disease problems at both the individual and population level. One application of this method is being undertaken to determine and validate syringe-sharing behavior among injection drug users by analyzing the biological residues in the syringes. Funded in part by DHHS #NO1-CO-12400.

Inferring relationships between pairs of individuals from locus heterozygosities. *S. Presciuttini*^{1,2}, *C. Toni*¹, *E. Tempestini*¹, *S. Verdiani*³, *L. Casarino*³, *I. Spinetti*¹, *F. De Stefano*⁴, *R. Dominici*¹, *J.E. Bailey-Wilson*². 1) Dept. Biomedicine, Univ. of Pisa, Italy; 2) NHGRI, Baltimore, MD, USA; 3) Dept. Medicina legale, Univ. of Genoa, Italy; 4) Ist. Medicina Legale, Univ. of Cagliari, Italy.

The traditional exact method for inferring relationships between individuals from genetic data is not easily applicable to some situations that may be encountered both in forensic science and in other disciplines. This study describes an approach that gives affordable results and is easily applicable; it is based on the probabilities that two individuals share 0, 1 or both alleles at a locus identical by state. We show that these probabilities [z(i)] depend on locus heterozygosity (H), and are scarcely affected by variation of the distribution of allele frequencies. This allows us to obtain empirical curves relating z(i) values to H for a series of common relationships, so that the likelihood ratio of a pair of relationships between any two individuals, given their genotypes at a locus, is function of a single parameter, H. Application to large samples of mother-child and full-sib pairs shows that the statistical power of this method to infer the correct relationship is not much lower than the exact method. Analysis of a large database of STR data proves that locus heterozygosity does not vary significantly among Caucasian populations, apart for special cases, so that the likelihood ratio of the more common relationships between pairs of individual may be obtained by looking at tabulated z(i) values.

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Hearing Loss in Fabry Disease: The Effect of Agalsidase Alfa Replacement Therapy. *L. Richfield, D. Hajioff, Y. Enever, R. Quiney, A.B. Mehta and Royal Free Hospital, London, U.K.* Clinical Haematology, Royal Free Hospital, London, London, United Kingdom.

HEARING LOSS IN FABRY DISEASE: THE EFFECT OF AGALSIDASE ALFA THERAPY LINDA RICHFIELD, DANIEL HAJIOFF, YVANNE ENEVER, ROBERT QUINEY, ATUL MEHTA. Aims We aim to describe the nature and prevalence of sensorineural hearing loss (SNHL) in Fabry disease and its response to enzyme replacement. Methods Fifteen men aged 25 to 49 (median 37) were randomised to placebo or -Agalsidase alfa (Replagal A replacement for six months, Transkaryotic Therapies Protocol TKT005) and have received enzyme replacement (Protocol TKT007) for a further 24 months so far. Audiological assessments were performed at 0, 6, 18 and 30 months. These included pure tone audiometry, transient-evoked otoacoustic emissions and impedance audiometry. Results Four patients (27%) had bilateral and seven (47%) had unilateral middle ear effusions with conductive losses persisting beyond six months. Three (20%) had normal hearing. At baseline, the median high frequency hearing loss (pure tone average air conduction at 4 kHz and 8 kHz) was 25 dB ISO (interquartile range: 15 49.4). This high frequency SNHL deteriorated over the first six months in both placebo and treatment groups by a median 4.3 dB ($p=0.002$, Wilcoxon matched pairs): the greater decline in the placebo group was not significantly different from the treatment group. This hearing loss subsequently improved beyond baseline by 2.1 dB at 18 months ($p=0.02$) and 5 dB at 30 months ($p=0.003$). High frequency bone conduction changed similarly. Baseline hearing losses correlated strongly with age (Spearman's $R_s=0.64$, $p=0.0001$) and glomerular filtration rate ($R_s=0.45$, $p=0.014$) but changes in hearing loss did not. Conclusions Eighty percent of adults with Fabry disease have significant hearing loss, usually high frequency SNHL. -Alalsidase alfa therapy appears to reverse the natural decline but this improvement is slow and small: long term study is required.

Hematopoietic cell transplantation (HCT) is effective treatment for mucopolidosis II (MLII) and a-mannosidosis (MAN). C. Peters^{1,2}, S. Grewal^{1,2}, P. Orchard^{1,2}, W. Krivit^{1,2}, S. Davies¹, E. Shapiro^{1,2}, R. Ziegler^{1,2}, L. Charnas^{1,2}, L. Lockman^{1,2}, K. Dusenbery², E. Braunlin^{1,2}, C. Milla^{1,2}, W. Regelman^{1,2}, S. Christiansen^{1,2}, F. Rimell^{1,2}, A. Petryk^{1,2}, A. van Heest², J. Ogilvie², M. Ahrens², S. Berry^{1,2}. 1) Depart. of Pediatrics; 2) Comprehensive Genetic Dis. Evaluation & Treatment Prog., Univ. of Minnesota, Minneapolis, MN.

MLII, a lysosomal enzyme localization disorder, exhibits progressive severe psychomotor delay, dysostosis multiplex and early death from cardiopulmonary complications. MAN demonstrates progressive psychomotor delay, craniosynostosis, ENT infections/hearing loss, organomegaly and shortened lifespan. We present our complete HCT experience in 3 pts with each disease; all are alive, fully engrafted with NL heart function. Diagnosis was established clinically/biochemically by deficient phosphotransferase activity and/or increased plasma levels of lysosomal enzymes (MLII) or by deficient a-mannosidase activity (MAN). HCT in MLII and MAN can halt cardiopulmonary and cognitive decline and promote a favorable QOL.

Patient	Age @ HCT	Donor	Follow-up	Verbal IQ	Perform.IQ	Pulmonary	QOL
MLII-1	1.6y	Related	4.7y	mod. delay	mod. delay	NI	Very good
MLII-2	0.3y	Related	1.5y	mod. delay	mod. delay	NI	Good
MLII-3	1.1y	Unrelated	1.5y	mod. delay	mod. delay	Pulm HTN	Fair/good
MAN-1	6.5y	Unrelated	5.4y	82	80	NI	Excellent
MAN-2	3.3y	Unrelated	5.1y	83	89	Mild restr	Excellent
MAN-3	23.1y	Unrelated	3.0y	?	?	NI	Good

Pamidronate use in infants with Osteogenesis Imperfecta (OI). *E.R. Elias*¹, *C.-H. Tsai*², *S. Zemel*³. 1) Special Care Clinic, Children's Hosp, Denver, CO; 2) Dept of Genetics, Children's Hosp, Denver, CO; 3) Dept of Endocrinology, Children's Hosp, Denver, CO.

The use of Pamidronate in OI has become common practice. However, its use in young infants, and the natural history of OI treated during infancy, is not well described. We report our experience treating infants with OI, two of whom developed severe neurologic complications.

Methods: Four infants with OI (2 girls and 1 boy with Type III-IV, and 1 girl with Type II-III) were treated with IV Pamidronate. A broviac catheter was placed prior to the first pamidronate course. Calcium metabolism was closely monitored pre and post-infusion. Urine calcium excretion and renal ultrasounds were assessed. Growth parameters and developmental course were monitored, and head imaging obtained.

Results: All four patients had early feeding difficulties and required neonatal oxygen. The child with Type II-III OI, now 26 months, is G-tube fed, with severe restrictive lung disease on O₂. Early nephrocalcinosis has resolved. Head MRI shows hydrocephalus, severe cervical stenosis, and basilar invagination. She is deaf, has moderate developmental delays, and has had line sepsis twice. She has occasional fractures. The male patient developed severe subdural hematomas and hydrocephalus 1 week after his first Pamidronate dose. Now 4 months, he has had no new fractures, but has continued feeding issues and needs O₂. The two other patients, now 8 and 9 months, are fracture-free, feed well, and have normal cognitive development.

Conclusions: Pamidronate can be safely used in young infants with OI. Broviac catheters ease blood draws and IV use, but line sepsis is a risk. Hydrocephalus was seen in 2/4 patients, with severe cervical stenosis and brainstem compression in 1, and subdural hemorrhage in the other. Clinical course is more helpful than biochemical results in defining prognosis in young infants with OI.

Bisphosphonate treatment for osteopenia associated with genetic disease in children. *S. Carter¹, R. Mendoza-Londono¹, V.R. Sutton¹, C.A. Bacino¹, K. Ellis², R. Shypailo², B. Lee¹.* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Dept. Pediatrics, CNRC, Baylor College of Medicine.

Osteoporosis is defined as low bone mass and bone fragility with increased susceptibility to fractures. It is also defined as bone mineral density (BMD) that is 2.5 SD below the mean peak value for age-matched controls. Until recently, treatment options for pediatric osteoporosis have been limited. Bisphosphonates inhibit osteoclast activity and have been shown to be safe in pediatric applications. Common genetic conditions associated with low BMD and susceptibility to fractures include osteogenesis imperfecta (OI), and secondary osteopenia due to lack of physical activity, as is seen in patients with neuromuscular disease. We present our experience treating 14 pediatric patients followed at the Texas Childrens Hospital Skeletal Dysplasia Clinic between 1999 and 2002. The diagnosis was OI type 3 or 4 in 11 patients and other neuromuscular disease in 3 patients. Ages ranged between newborn and 15 yrs. (Average 4.5 yrs). The diagnosis of OI was made in the first two months of life in 9 of the 11 patients. Bone mineral density of the lumbar spine, femoral head and total body was measured using dual-energy x-ray absorptiometry (DXA) before treatment and at regular intervals during treatment. The patients received Pamidronate at a dose of 13.5 mg/kg/year for children <2-3 years and 9 mg/kg/year for children >3 years. Bone metabolic parameters (alkaline phosphatase, urine collagen N-telopeptides) showed decrease in bone turnover. Average L-spine BMD values were Z= -3.86 (Range 6.78 to 2.92) before treatment and Z= -2.56 (range 3.72 to 1.4) after treatment. Physiologic response appeared more significant in younger children. Overall there was a subjective clinical improvement, with decrease in bone pain and frequency of fractures, resulting in better tolerance of daily activities and well being. Side effects were minor including transient febrile episodes and mild hypocalcemia which corrected with supplementation. This experience has demonstrated that bisphosphonates constitute an important adjuvant treatment for osteoporosis associated with genetic disease.

Precocious Puberty during Outpatient Pamidronate therapy in type I Osteogenesis Imperfecta. *T.C. Markello¹, B. Boston², R. Steiner³*. 1) Dept Pediatrics, Samaritan Health Services, Lebanon, OR; 2) Pediatrics, Oregon Health Sciences University, Portland, OR; 3) Molecular and Medical Genetics, Oregon Health Sciences University, Portland, OR.

Intermittent iv bisphosphonate therapy has decreased pain and reduced fractures in children with type I Osteogenesis Imperfecta (Glorieux et. al., NEJM 339:947-52,1998). We describe the clinical course, and one unique complication during the treatment of two sisters over 3 years. Methods: After informed consent and local IRB approval, treatment using bisphosphonate therapy was begun. The protocol followed published methods, modified to the outpatient environment of a rural pediatric practice. Results: Type I OI was confirmed by collagen fibroblast analysis in an affected first cousin. Prior to therapy both siblings had mild extremity fracture rates of 1 to 2 per year. Both had vertebral compression fractures by 2 years old, consistent with severe spinal involvement, a known variation in this familys OI expression. No fractures have occurred in either child since starting therapy. Growth in vertebral body height has improved in areas of previous compression fractures. One child began adrenarche 10months after beginning iv pamidronate, at age 7.0yr. By age 8.5yrs she showed an advanced bone age of 11years, elevated gonadotropins and breast development. At that time she was begun on dual therapy with pamidronate and leuprolide and has not had progression of precocious puberty. Her sister has not shown similar signs of precocious puberty after 30 months of therapy, but is currently only 5.5yr old. No other family member in the extended pedigree, with or without OI, has been documented to have precocious puberty. Conclusions: This is the first reported case of precocious puberty while receiving bisphosphonate therapy. It may be coincidental, or a rare complication. It did not require discontinuation of treatment nor has her sibling shown similar changes.

Controlled trial of pamidronate in children with Types III and IV OI. *J.C. Marini¹, A.D. Letocha¹, J. Reynolds³, C.M. Reing⁶, E. Chernoff¹, J. Troendle², S. Hill⁴, L. Gerber⁵, F.H. Glorieux⁷.* 1) SCTD/ NICHD/NIH, Bethesda, MD; 2) BMSB/NICHD/NIH, Bethesda, MD; 3) NMD/CC/NIH, Bethesda, MD; 4) DRD/CC/NIH, Bethesda, MD; 5) RMD/CC/NIH, Bethesda, MD; 6) Dept Ortho, INOVA, Fairfax, VA; 7) Shriners, Montreal, Quebec.

We are engaged in a randomized, non-blinded controlled trial of pamidronate (Aredia®, Novartis) in children with types III and IV OI. The purpose was to assess the effects of intermittent inhibition of bone resorption on vertebrae, long bones and serum markers of bone matrix and growth in children with abnormal matrix. Children over 2 years are randomized to receive pamidronate or not. Children are seen every 3 months; the pamidronate group receives 30 mg/m² over 3 days. Every visit includes physical rehabilitation and measurement of serum markers. AP and lateral x-rays of the spine and lower long bones and spine DEXA are obtained at 0, 6 and 12 months. QCT scan of the lumbar spine and femoral mid-shaft is obtained at 0 and 12 months. Dual-labeled iliac crest biopsies are taken at 0 and 12 months for histomorphometry. Fifteen patients completed 1 year; 2 completed 6 months. Repeated measures analysis examined 9 pamidronate vs 8 no-pamidronate patients. Baseline, 6 and 12-month vertebral data and baseline, 3, 6, 9, 12, 15 and 18-month data for serum markers were analyzed. Bowing, MOI, fracture rate and histomorphometry are pending. Pamidronate treatment resulted in significant differences for vertebral BMD, compression ratio and area. L1-L4 DEXA z-scores increased significantly for the pamidronate group vs controls (p=0.046). Vertebral compressions significantly improved in pamidronate vs no-pamidronate groups at T12, L1 and L2 (p=0.017, 0.038 and 0.006). Vertebral area significantly improved in treatment vs control groups at L1 and L2 (p=0.041 and 0.053) but not T12 (p=0.092). Analysis of serum markers of bone synthesis (PICP and OC) showed no significant difference between groups. These data support the claim that pamidronate enhances vertebral BMD and resistance to compression in children with OI. It remains unclear whether pamidronate is functionally beneficial or detrimental for cortical bone in the extremities.

Treatment of spinal muscular atrophy by hydroxyurea. *J.G. Chang¹, F.J. Tsai¹, W.Y. Wang², Y.J. Jong³.* 1) Dept Medical Res, Div Molec Med, China Medical Col Hosp, Taichung, Taiwan; 2) School of Rehabilitation Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; 3) Departments of Pediatrics and Clinical Laboratory, Kaohsiung Medical University, Kaohsiung, Taiwan.

Background Proximal spinal muscular atrophy (SMA) is a degenerative motor neuron disease caused by a deficiency of the functional SMN protein. Effective treatment for this disorder is unavailable at present. We evaluated the effect of hydroxyurea treatment in patients with this disorder. **Methods** We treated different types of SMA cell lines with hydroxyurea to evaluate the effect on SMN (SMN2) expression, and then treated 2 of type I, 5 of type II and 2 of type III SMA patients with hydroxyurea at a dose of 35 mg per kilogram of body weight daily for 10 months. The patients were evaluated at baseline and every month for 10 months by detailed clinical examinations, muscle test, clinical laboratory evaluation, and measurements of expression of SMN gene. **Findings** The expression of full-length mRNA of the SMN2 gene increased significantly in all three types of SMA lymphoblastoid cell lines and in the blood mononuclear cells of SMA patients. Muscle power and clinical symptoms also improved. The effect began 2 months after treatment, and maximal effect was found within 4-6 months of treatment. No definite side effects were found in this study. **Interpretation** Hydroxyurea increases full-length mRNA expression of the SMN 2 gene both in vitro and in vivo, and ameliorate clinical manifestations of SMA patients. This drug offers a new approach in the treatment of SMA disease.

Performance IQ (PIQ) and MRI Severity Score (MRI-SS) Predict Survival and Disability after Hematopoietic Stem Cell Transplantation (HSCT) for Childhood Onset Cerebral Adrenoleukodystrophy (CALD). *L. Charnas, E. Shapiro, W. Krivit, L. Lockman, R. Ziegler, S. Abel, S. Grewal, P. Orchard, C. Peters.* Department of Pediatrics, University of Minnesota, Minneapolis, MN.

Introduction: Adrenoleukodystrophy (X-ALD) is an X-linked peroxisomal disorder characterized by impaired very long chain fatty acid (VLCFA) oxidation. 35% of boys with X-ALD will develop childhood onset cerebral disease (CALD) with cerebral demyelination, neurodegeneration and death 3-5 years after onset of symptoms. HSCT arrests disease progression in early CALD, but outcome in more advanced CALD is less well understood. The morbidity and mortality of HSCT are too great to offer to boys with biochemical X-ALD but no evidence of CALD. **Methods:** Between 1990 and 2001, 110 boys with XALD were evaluated at the University of Minnesota (UMN). 51 underwent HSCT at UMN, 10 were offered HSCT but were not treated at UMN, 18 had no cerebral disease, and 31 were too severe at presentation to be considered for HSCT. All had brain MRI, testing of verbal (VIQ) and PIQ and neurologic examination. An MRI-SS was assigned to each MRI. **Results:** Of the 51 boys undergoing HSCT, 23 boys had mild/moderate disease (PIQ>80, MRI-SS 0.5-14). 15 are alive, all can see and function independently. Only one death occurred due to ALD progression. 28 boys with severe disease (PIQ < 80, MRI-SS 5-20) had HSCT. 12 are alive, including 3 recent transplants. All are disabled, 5 are blind. ALD progression caused 11 deaths. A low MRI-SS was the best predictor of survival (P<0.001). PIQ and VIQ did not predict survival. Boys starting with a PIQ>80 had no significant functional deficits. Survivors with initial PIQ<80 had significant functional deficits, usually severe or profound. Initial MRI-SS and PIQ were inversely correlated, R = -.85. Both MRI-SS and PIQ were strongly correlated with the ALD Disability Rating Scale score, R = 0.65 and R = 0.85, respectively. **Conclusion:** MRI-SS identifies boys with early CALD and predicts survival from HSCT. PIQ predicts disability following HSCT. PIQ < 80 identifies boys at high risk for poor outcomes with current methods. HSCT is an effective therapy for boys with early, but not advanced, CALD.

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Pancreatic cancer therapy by intratumoral gene transfer of IL-13Ra2 chain followed by IL-13R-targeted bacterial cytotoxin administration in animal models. *K. Kawakami, M. Kawakami, S.R. Husain, R.K. Puri.*
Laboratory of Molecular Tumor Biology, Division of Cellular and Gene Therapies, CBER/FDA, Bethesda, MD.

Interleukin-13 receptor (IL-13R) $\alpha 2$ chain plays a key role in ligand binding and internalization. We have recently demonstrated that this cytokine receptor chain has unique characteristics in tumor biology: it inhibits tumorigenicity of breast and pancreatic cancer in animal models. In this study, we have utilized this chain and established a novel approach for aggressive pancreatic cancer therapy. For this, a plasmid containing IL-13Ra2 chain gene was mixed with liposome (a mixture of DOTAP and cholesterol) and injected into subcutaneously or orthotopically xenografted human pancreatic tumors in immunodeficient mice, followed by systemic or local therapy by IL-13 cytotoxin. IL-13 cytotoxin is a fusion protein comprised of human IL-13 and a truncated form of bacterial toxin (*Pseudomonas* exotoxin). Only tumors forced to express IL-13Ra2 chain acquired extreme susceptibility to the antitumor effect of IL-13 cytotoxin. There was a dominant infiltration of cells including macrophages and natural killer cells in the regressing tumors. Because macrophages were found to produce nitric oxide, IL-13Ra2 targeted cancer therapy involved not only a direct tumor cell killing by IL-13 cytotoxin but also activation of innate immune response at the tumor site. Therefore, this approach may be a new powerful tool for pancreatic cancer or other localized cancer therapy.

Enzyme Replacement Therapy (ERT) with agalsidase alfa leads to Regression of Left Ventricular Hypertrophy in Male and Female Patients with Anderson Fabry Disease (AFD). C. Kampmann¹, F.A. Baehner², C. Martin¹, C.M. Wiethoff¹, C. Whybra², E. Miebach², M. Ries², M. Beck². 1) Pediatric Cardiology, University Children's Hospital, Mainz, Germany; 2) Dpt. of Lysosomal Storage Diseases, University Children's Hospital, Mainz, Germany.

Background: In both genders, AFD is accompanied by a progressive hypertrophic cardiomyopathy (CM), which is related to accumulation of globotriaosylceramide (Gb3) in the myocardium. ERT is now available in Europe. Purpose of the study was to evaluate the effect of agalsidase alfa on the AFD-related CM in both males and females. **Methods and Results:** 40 genetically proven hemi- and heterozygotes (22 males, aged 34.5 ± 9.3 years and 18 females, aged 45.3 ± 12.8 years), older than 18 years, received biweekly 0.2 mg/kg BW infusions of agalsidase alfa (Replagal®). Echocardiographic and electrocardiographic examinations were performed at baseline and at three monthly intervals. 6 mo. data were available in 34 pts, 9 mo. data in 16, 12 mo. data in 9 and 18 mo. data in 2 pts. Overall, there was a progressive decrease in left ventricular wall thickness over time ($F=30.20$; $p=0.0001$) and also a progressive decrease in LV mass index (baseline: 172 ± 61.3 g/m², 3 mo: 160.5 ± 56.4 g/m², 6 mo: 145 ± 39.5 g/m², 9 mo: 122.5 ± 37.1 g/m², 12 mo: 121 ± 33 g/m²; $F=13.82$; $p=0.003$). QRS duration ($F=4.34$; $p=0.039$) and ECG signs of LV hypertrophy: Sokolow-Lyon voltage-duration product ($F=4.98$; $p=0.028$) also decreased progressively with ERT. No patient worsened during treatment. All patients in NYHA functional class III or IV (13 pts (6 males and 7 females) improved at least one class during therapy. **Interpretation:** ERT with agalsidase alfa in patients with hypertrophic CM related to AFD leads to significant reductions in LV wall thickness and mass, which are progressive with long term therapy, with associated improvement in NYHA clinical status in those patients with moderate to severe heart failure, presumed secondary to diastolic LV dysfunction. **Conclusion:** Agalsidase alfa (Replagal®) is effective in regressing AFD related hypertrophic CM in both male hemizygotes and female heterozygotes.

Vascular smooth muscle specific gene expression in vivo by the fiber-modified adenovirus vector. T.

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Vascular smooth muscle cells (SMCs) are an important target for gene therapy of atherosclerosis and restenosis. We are also interested in the possible involvement of vascular SMCs in progression of Duchenne muscle dystrophy (DMD). Specific gene expression in vascular SMCs may be important for elucidating the pathogenesis of DMD. In the present study, we attempted to establish the vascular SMC specific gene expression system which can be used for in vivo gene transfer. We constructed the hybrid promoter composed of the basic promoter of SM22alpha which is expressed exclusively in SMCs and the immunoglobulin heavy chain intron 1 enhancer. The hybrid promoter significantly increased the promoter activity with remaining the SMC specificity. Vascular SMCs express very low levels of the primary adenovirus receptor, coxsackie virus and adenovirus receptor (CAR). To increase the adenovirus mediated transduction, we generated a fiber-modified adenoviral vector containing the Arg-Gly-Asp (RGD) peptide that may infect the cells through the interaction of RGD and integrin avb5. The fiber modified adenovirus vector showed 10 times more efficient gene transfer into vascular SMCs in vitro. Finally, we directly administered this adenoviral vector to uninjured rat femoral arteries. The high expression of reporter gene was observed only in artery SMCs of the tunica media but not in endothelial or adventitial cells. Taken together, our results demonstrate that the fiber-modified adenoviral vector containing the SM22alpha based hybrid promoter may provide a highly effective tool for gene therapy of vascular disease.

Monitoring of Gene Stability Following Targeted Receptor-Mediated Endocytosis of the pAI-1 Insulin-Plasmid in HepG2 Cells. *L.R. Wing¹, H.S. Hamlin¹, M.A. Marino²*. 1) Dept Biopharmaceuticals, Transgenomic, Inc, San Diego, CA; 2) Transgenomic, Inc, Gaithersburg, MD.

Non-viral, receptor-mediated gene-delivery systems have been used to transfer genes into target cells followed by expression of the gene products of therapeutic importance. Targeted receptor-mediated pathways include the addition of targeting ligands to the DNA to induce specific binding, such as synthetic or naturally occurring carbohydrate ligands. Investigation of galactose-coupled plasmid DNA vectors following intracellular uptake and internalization includes the induction of mutations in the gene of interest. Depurination and other base pair alterations may occur due to the low pH and hydrolase activity in lysosomal storage vesicles, resulting in truncated, aberrant proteins with no activity or potential toxicity. Gene stability by mutation detection of the galactose-coupled pAI-1 plasmid (pBR322 containing the insulin gene) was evaluated following uptake, intracellular transport and lysosomal storage in HepG2 cells by denaturing HPLC using the WAVE® DNA Fragment Analysis System (Transgenomic, Inc.). Following the binding of pAI-1 to HepG2 cell surfaces (2h, 4⁰C) and endocytosis (0-2h, 37⁰C), pAI-1 was isolated from the culture media and cell lysate in the presence and absence of chloroquine (an inhibitor of endocytosis). A 240bp PCR product containing the insulin gene was isolated from the HepG2 media and lysate at various time points following the initiation of endocytosis (0,15,30,45,60,90,120 min). Samples were mixed with reference wild-type fragment to generate homoduplexes and/or heteroduplexes and subjected to dHPLC. The data indicated the formation of heteroduplexes in the lysates of the untreated HepG2 samples after 30 minutes post-endocytosis, whereas the chloroquine-treated HepG2 samples demonstrated only homoduplex peaks for each time point in the media and lysates. These results suggest that the process of receptor-mediated endocytosis may play a role in the generation of mutations in DNA-based therapeutics and intracellular gene stability monitoring of therapeutic genes is essential for evaluating the success of gene therapy delivery systems.

Mechanism of acid sphingomyelinase uptake by Niemann-Pick disease macrophages: Implications for enzyme replacement therapy. *R. Dhami*¹, *K. Kary*², *E.H. Schuchman*¹. 1) Dept Human Genetics, Mount Sinai Sch Medicine, New York, NY; 2) Genzyme Corporation, Cambridge, MA.

Types A and B Niemann-Pick disease (NPD) result from deficient acid sphingomyelinase (ASM) activity. Type A NPD is characterized by early onset neurodegeneration that leads to death by ~3 years of age, whereas patients with Type B NPD have little or no neurological involvement and may survive into adulthood. A mouse model of Types A and B NPD (ASMKO mice) has been constructed that develops many of the same biochemical, pathological, and clinical abnormalities as human NPD patients. To gain a better understanding of the mechanisms underlying ASM uptake by NPD macrophages, we obtained alveolar macrophages from normal and ASMKO mice and evaluated the uptake of recombinant human enzyme obtained from the media of overexpressing CHO cells. Two forms of recombinant enzyme were utilized: standard ASM containing complex oligosaccharides (cASM), and a modified form of ASM that contained high mannose type oligosaccharides (mASM). Surprisingly, both forms of recombinant ASM were taken up poorly by ASMKO macrophages as compared to normal macrophages, although the efficiency of uptake of mASM was ~10-fold greater than cASM. Inhibitor studies revealed that in both normal and mutant cells, mASM was primarily internalized by mannose receptors, while cASM was internalized by both mannose and mannose-6-phosphate receptors. To investigate why NPD macrophages internalized ASM poorly, receptor recycling studies were undertaken. These experiments revealed a general recycling defect in the NPD macrophages. Thus, a hypothesis is proposed to explain the therapeutic effectiveness of enzyme therapy in the lungs of NPD mice that suggests that the effects are largely due to a reduction in macrophage recruitment into the lungs, rather than direct enzyme uptake by lung macrophages.

Ambulatory enzyme replacement therapy and clinical evaluation in Fabry's disease. *F. Breunig¹, F. Weidemann¹, J. Strotmann¹, M. Beer², V. Krane¹, C. Wanner¹.* 1) Department of Nephrology, University Clinic, Wuerzburg, Germany; 2) Department of Radiology, University Clinic, Wuerzburg.

Fabry disease is X-linked lysosomal storage disorder due to α -galactosidase A (α -GAL A) deficiency with intracellular accumulation of the incomplete metabolised substrate, mainly globotriaosylceramide (Gb3). Affected organs are the vascular endothelium, heart, brain and the kidney with progressive renal insufficiency. We investigated feasibility, safety and side effects of ambulatory enzyme replacement therapy (ERT) in Fabry patients. Furthermore we established a clinical evaluation program to document baseline status and the course of the disease during ERT. Ongoing clinical evaluation includes cardiac testing, kidney and pulmonary function as well as dermatological evaluation. Recombinant α -GAL A (Agalsidase b, Fabrazyme®) was given i.v. to 16 male and 2 female patients every two weeks in a dosage of 1mg/kg/bw. Mean age was 42,4 years (range 29 to 57). Mean duration of treatment is 8,2 months (range 3-12). Infusion time was reduced within 8 applications from 240 to 150 min.. Patients were pretreated with paracetamol, clemastin and ranitidin to prevent infusion related reactions. So far about 300 infusions were administered. Documented side effects were fatigue after therapy (frequently), chills without fever (three patients), rigor (one patient), cramps of the calves (one patient, agalsidase b was administered during hemodialysis) and vertigo (one patient, during two non successive infusions). One patient had to start hemodialysis after two months of ERT (baseline creatinin clearance 7ml/min). In general treatment was well tolerated, no serious side effects occurred. All patients were treated as outpatients. No increased occurrence of side effects at a infusion time of 150 min compared to 240 min.. We conclude that ambulatory ERT of Fabry's disease with recombinant α -GAL A is feasible and safe. Side effects and clinical course at baseline and during therapy should be well documented. To reveal the clinical benefit of long term ERT on critical organs like kidney, heart and brain comprehensive clinical evaluation programs should be conducted on a regular basis.

The effect of enzyme replacement therapy with agalsidase alfa on cerebrovascular disease in a female heterozygote with Fabry disease. *J.T.R. Clarke*^{1, 2}, *C. Jaigobin*², *J.R. Wherrett*², *R.M. Iwanochko*³. 1) Clinical Genetics, Hosp Sick Children, Toronto, ON, Canada; 2) Neurology, University Health Network, Toronto, ON, Canada; 3) Nuclear Cardiology, University Health Network, Toronto, ON, Canada.

Neurological complications are common in both hemizygous males and heterozygous females with Fabry disease. Transient ischemic attacks (TIAs) and strokes (CVAs) are reported to occur in at least 25% of hemizygous males over 18 yrs of age and has also been reported in heterozygous females. Treatment of Fabry disease by enzyme replacement therapy (ERT) with agalsidase alfa has been reported to ameliorate the pain and reverse cerebral circulatory abnormalities, but clinical benefit in the treatment of TIAs has not been confirmed. Aim: We wish to report on our experience with ERT in a female Fabry heterozygote who presented with multiple TIAs at 19 yrs of age. Methods: The diagnosis of Fabry disease was established by demonstration of (1) typical intralysosomal inclusions in blood vessels in muscle, (2) elevation of ceramide trihexoside (CTH) levels in plasma, and (3) the presence of a missense mutation, 776C>T (P259L), in the *GLA* gene. Plasma a-galactosidase A levels were normal. After baseline clinical neurological investigation and cerebral MRI scanning, the patient underwent ERT by biweekly IV infusions of agalsidase alfa, 0.2 mg/kg. The effect of treatment was assessed by repeating the baseline studies after 15 months of ERT. Results: In the year prior to starting ERT, the patient suffered frequent recurrent headaches and had 3 transient episodes of dysarthria and left hemiparesis associated with the development of MRI lesions in the basal ganglia and the pons. Within 6 months of starting ERT, the headaches remitted and she had no further TIAs. Follow-up neurological examination showed no focal neurological deficits, though deep tendon reflexes were generally increased. Conclusions: Experience with this patient showed that (1) TIA is a potentially serious vascular complication of Fabry disease, even in young heterozygous females; and (2) the frequency and severity of TIAs is decreased by ERT with agalsidase alfa.

Biodistribution and cellular localization of recombinant α -galactosidase A: A comparison of Fabrazyme® and Replagal™. *T. Edmunds, P. Piepenhagen, L. Copertino, C. Nickerson, L. Andrews.* Genzyme Corporation, Framingham, MA.

Fabry disease is a lysosomal storage disease arising from deficiency of the enzyme α -galactosidase A. Two protein therapeutics, Fabrazyme (α -galactosidase beta) and Replagal (α -galactosidase alfa), have been approved as enzyme replacement therapies for Fabry disease in Europe and are undergoing further clinical testing in the United States. Both products contain the same protein, human α -galactosidase A. Fabrazyme is produced in a CHO cell line while Replagal is produced in a human cell line. Since these two production systems could lead to different post-translational modifications we have carried out biochemical comparisons of the two products. Biochemical analyses have demonstrated that the two products are structurally similar and functionally equivalent with the structural variations limited to the degree of sialylation and phosphorylation of oligomannose chains. Fabrazyme has a higher sialic acid to galactose ratio and a higher level of mannose-6-phosphate per mole of protein. While these variations in glycosylation do not significantly affect the in-vitro properties of the proteins they could impact biodistribution at either the organ or cellular level. To answer this question we conducted biodistribution and cellular localization studies in Fabry mice. Biodistribution studies were carried out in Fabry mice given equivalent (3mg/kg)i.v. bolus doses of each enzyme. Enzyme activities were measured in the kidney, heart, liver and spleen 1, 4, 8 and 24hr following injection. Cellular localization within these organs was determined using enzymes fluorescently labeled with either Oregon Green-488 or Alexa Fluor-546.

These studies demonstrated that similar amounts of each product are delivered to the tested organs with the majority of the injected dose (66% for Fabrazyme, 69% for Replagal) recovered in the liver 1hr following injection. Lower amounts of each enzyme were recovered from the kidney, heart and spleen. These results indicate that the variations in the glycosylation of the two products do not have a significant affect on organ distribution.

RhIDU enzyme replacement therapy for MPS I: 24-week extension study. *L.A. Clarke*¹, *J. Muenzer*², *E.H. Kolodny*³, *G.M. Pastores*³, *M. Beck*⁴, *J.E. Wraith*⁵. 1) UBC, Vancouver, BC, Canada; 2) UNC, Chapel Hill, NC; 3) NYU, NY, NY; 4) UMainz, Mainz, Germany; 5) UManchester, Manchester, UK.

Objective: To further evaluate the safety and efficacy of recombinant human α -L-iduronidase (rhIDU) for treatment of MPS I. **Methods:** All 45 patients from the 26-week Phase 3 randomized, double-blind, placebo-controlled study (Ph3DB) entered an open-label extension study (Ph3EXT) and received 100 U/kg (=0.58 mg/kg) rhIDU IV weekly. Efficacy endpoints were evaluated by within-group changes (rhIDU/rhIDU, n=22, 50 weeks rhIDU treatment; placebo/rhIDU, n=23, 24 weeks rhIDU treatment) from baseline (start of Ph3DB) and entry (start of Ph3EXT) to Week 24 of the Ph3EXT. **Results:** At baseline, the mean age was 15.5 years (range 6-43) and 82% had Hurler-Scheie syndrome (moderate phenotype). After 50 weeks of treatment, rhIDU/rhIDU patients maintained the improvement in percent of predicted normal FVC made in the Ph3DB (5.9 percentage points, p=0.003) and improved further in the 6MWT (42.9 meters, p=0.005). Placebo/rhIDU patients showed a slight decline in FVC in both the Ph3DB and PhEXT (-0.6 percentage points) but improved in the 6MWT by 23.8 meters (p=0.073), similar to rhIDU patients in both the Ph3DB and Ph3EXT. Placebo/rhIDU patients showed large reductions in liver volume (-12.6%) and urinary GAG level (-68.9%) comparable to those achieved and maintained by rhIDU/rhIDU patients. In patients with apnea-hypopnea index scores suggestive of sleep apnea, rhIDU/rhIDU patients maintained improvement seen in the Ph3DB, while placebo/rhIDU patients also improved. Increased joint range of motion and improved quality of life scores were observed in both groups. The safety profile was acceptable and similar to that of the Ph3DB. One patient death, unrelated to study drug, occurred. As with rhIDU-treated patients in the Ph3DB, nearly all patients (21/23) in the placebo/rhIDU group developed anti-rhIDU IgG antibodies; 2 patients in the rhIDU/rhIDU group tolerized. Antibody formation did not appear to impact safety or efficacy. **Conclusion:** rhIDU treatment continues to appear to be a promising treatment for the non-CNS components of MPS I disease. (Sponsored by Genzyme and BioMarin).

Enzyme replacement therapy improves cardiovascular control in Fabry patients during orthostatic stress. *M.J. Hilz*^{1, 2}, *H. Marthol*¹, *M. Brys*¹, *T. Haendl*², *G. Welsch*², *R. Franta*², *B. Stemper*^{1, 2}. 1) Neurology, New York University, New York, NY; 2) Neurology, University of Erlangen-Nuremberg, Erlangen, Germany.

Fabry disease, an X-linked α -galactosidase A deficiency, results in glycolipid accumulation in various organs including the nervous system. Loss or dysfunction of small nerve fibers may impair cardiovascular autonomic modulation. Although enzyme replacement therapy (ERT) with recombinant human α -galactosidase A has recently become available in many countries, the effects of ERT on autonomic cardiovascular control have not been analyzed. In this study, we evaluated whether ERT improves cardiovascular control during orthostatic stress in Fabry patients. In 11 Fabry patients (28.4 ± 7.9 years) undergoing ERT (1mg/kg recombinant human α -galactosidase A, Fabrazyme every two weeks) for 23 months and in 11 patients (28.8 ± 9.1 years) undergoing ERT for 18 months, we monitored electrocardiographic RR-interval (RRI) and blood pressure (BP) [Colin Pilot] at supine rest and during 5 minutes of active standing before and after ERT. As parameters of autonomic control, we analyzed spectral powers of RRI and BP in the low (LF: 0.04-0.15 Hz) and high (HF: 0.15-0.5 Hz) frequency range using autoregressive analysis. Differences between parameters measured in supine and standing position were expressed as percentage changes. After ERT, the orthostasis induced heart rate acceleration, i.e. decrease in RRI was more pronounced ($-28.4 \pm 13.2\%$) than before therapy ($-4.9 \pm 7.1\%$; $p < 0.05$). ERT had no influence on orthostasis induced changes of BP or RRI-LF-power ($p > 0.05$). In contrast, the orthostatic reduction of parasympathetic HF-power of RRI was more prominent after ERT ($-79.8 \pm 23.1\%$) than before therapy ($-46.7 \pm 58.1\%$; $p < 0.05$). After ERT, the orthostasis induced increase in sympathetic LF-power of BP was higher (357.9 ± 349.4) than before therapy ($112.7 \pm 177.1\%$; $p < 0.05$), while HF-power of BP remained unchanged. To conclude, ERT improves orthostatic cardiovascular control in Fabry patients as demonstrated by increased orthostatic heart rate acceleration due to cardiovascular withdrawal, sympathetic activation and sympathetic vasomotor control following therapy.

A phase I/II clinical study evaluating the safety and clinical activity of enzyme replacement therapy in Mucopolysaccharidosis II (Hunter syndrome). *J. Muenzer, D. Towle, M. Calikoglu, S. McCandless.* Department of Pediatrics, University of North Carolina, Chapel Hill, NC.

Mucopolysaccharidosis II (MPS II) is an X-linked recessive disorder caused by the deficiency of the lysosomal enzyme iduronate-2-sulfatase (I2S). MPS II is a progressive disorder with tissue and organ involvement due to storage of glycosaminoglycans (GAG). There is no effective therapy for MPS II. Exogenous enzyme replacement therapy offers the possibility of a safe and efficacious approach for the treatment of the somatic disease in MPS II. Human I2S was produced by genetic engineering technology in a human cell line by Transkaryotic Therapies (TKT), Cambridge, MA. Preclinical studies in an MPS II knock-out mouse model have demonstrated that treatment with human I2S results in decreased urinary GAG and reduction in tissue GAG. The phase I/II enzyme replacement clinical trial started in March 2001 with the primary goal of the trial to determine safety of the genetically engineered enzyme and the secondary goal to determine clinical activity. The clinical trial was a randomized, double-blind, placebo-controlled clinical study which enrolled twelve MPS II patients (6 to 20 years of age). Three dosages of I2S (0.15 mg/kg, 0.5 mg/kg and 1.5 mg/kg) were studied. Within each dose group, patients were randomized to receive either I2S (three patients) or placebo (one patient) by IV infusion biweekly for six months. All twelve patients have successfully completed the six-month study and all elected to enroll in an open-label extension study. Preliminary analyses of the data showed a 51% reduction in urinary GAG excretion in the MPS II patients receiving I2S. I2S treatment was also associated with significant reductions in liver and spleen volumes. Infusion-related reactions have occurred in four patients in the middle and high dose groups and have been successfully managed by slowing the infusion rate and using premedications. These initial results indicate that this I2S preparation is clinically active and is a promising treatment for patients with MPS II.

Replagal Treatment in Fabry Patients Following Renal Transplantation. *K. Nicholls, E. Centra, B. Lehmann, G. Becker.* Nephrology, Royal Melbourne Hospital, Parkville, Australia.

AIMS: To study the safety, tolerability and effects of Replagal on clinical findings, quality of life, renal function and exercise capacity in male Fabry patients with functioning renal transplants. **PATIENTS AND METHODS:** 4 patients aged 59, 59, 43 and 31 years received compassionate use Replagal fortnightly for 18 months. Patients had undergone successful renal transplantation 2.5-17 years previously, and had stable renal function. The two oldest patients were significantly disabled by advanced symptoms of Fabry disease, particularly muscle fatigue on minimal exertion. Patients kept daily diaries of exercise, bowel habit and all medication. Study visits at Weeks (W) 0-1, 9, 17, 24 and months (M) 9, 12 15 and 18 documented clinical findings, SF36 and EQ5D questionnaires, hematology and biochemistry (renal & liver function, lipids, albuminuria). Prophylactic analgesics were stopped prior to each study visit. Radionuclide glomerular filtration rate (GFR) was performed at baseline then 6-monthly. **RESULTS:** Tolerability: 3 patients tolerated infusions well, and 1 developed moderate infusion reactions from 12 months, requiring pre-infusion corticosteroid and antihistamine. Sweating: Patients reported definite increase in sweating at W9, 17, 17 and 37. Pain: One of the 3 patients on prophylactic carbamazepine was able to stop at M9, one decreased dose progressively at W9, M12 and M13, while the third maintained dose with decrease in pain score over M3-12. Exercise: Patients reported significantly increased daily exercise at 12 months compared with baseline (Scored as mean daily score over monthly intervals, ANOVA $p < .05$). Bowel habit: No change in bowel habit was seen. Renal Function: GFR, creatinine clearance and proteinuria remained stable. **CONCLUSIONS:** Over 72 patient months, Replagal was well tolerated, and was associated with increased sweating, decreased pain and increased physical activity.

Gastrointestinal symptoms in Fabry's disease respond to enzyme replacement therapy. *B.E. Hoffmann, B. Koletzko.* Div. of Metabolic Diseases & Nutrition, Dr. von Hauner Children's Hospital, Munich, Germany.

INTRODUCTION

M. Fabry is an X-linked metabolic disorder with autosomal recessive inheritance, deficiency of α -galaktosidase A and localisation of the defect at position Xq22.1. Globotriasylceramide (Gb3) accumulates in several tissues causing unlikely clinical findings (neuropathic pain, angiokeratoma, abnormal renal function, cardiomyopathy, gastrointestinal symptoms etc., 1,2). Reports about gastrointestinal symptoms in M. Fabry are few and no information has been published on possible response of gastrointestinal symptoms to enzyme replacement therapy (ERT).

CASE REPORT

Acroparaesthesia showed up at the age of 6 years and got worse with physical exercise and elevated temperature. From 10 years onwards symptoms became more severe and exacerbated by consumption of different foods. 18 years old determination of α -Galaktosidase-A (activity of 8 percent) confirmed the diagnosis of M. Fabry. Treatment was symptomatic. 34 years old gastrointestinal symptoms had become leading complaints (constipation and diarrhoea, dyspeptic symptoms and early feeling of fullness). Only small food portions within 10 meals/d were tolerated but body weight was only 51.2 kg, length 176.6 cm and BMI 16,4 kg/m.

ERT was initiated with a biweekly dose of 0.2 mg/kg of human recombinant α -galaktosidase (Agalsidase alfa). Medication was tolerated well except for a mild headache several times. Gastrointestinal complaints improved after a few infusions, the feeling of fullness waned, larger meal portions and a smaller meal frequency were tolerated. After 9 months of treatment BMI was increased to 17,4 kg/m.

CONCLUSIONS

Fabry's disease may present with severe gastrointestinal symptoms suggestive of a motility disorder that induce malnutrition. ERT with agalsidase alfa reduced these symptoms, made metoclopramide superfluous, increased body weight and BMI and enhanced life-quality. 1 Mac Dermot et al. 2001; 2 Argoff et al. 1998.

Treatment of Classical Infantile Pompe Disease(CIPD) with Recombinant Human Acid Alpha

Glucosidase(rhGAA): Preliminary 6 month data from a Phase 2 study. P. Kishnani¹, T. Voit², M. Nicolino³, A. Amalfitano¹, C.H. Tsai⁴, G. Herman⁵, J. Waterson⁶, R.C. Rogers⁷, H. Landy⁸, D. Corzo⁸, B. Thurberg⁸, S. Richards⁸, F.H. Yong⁸, Y.T Chen¹. 1) Dept of Medical Genetics, Duke University Medical Center, Durham, NC; 2) Univ Hosp, Essen, Ger; 3) Ped Hosp Brousse, Lyon, Fr; 4) Children's Hosp Denver, CO; 5) Children's Hosp Columbus, OH; 6) Children's Hosp Oakland, CA; 7) Greenwood Genet Cr, Greenville, SC; 8) Genzyme Corp, USA.

Purpose: To evaluate the safety and efficacy of rhGAA in patients with CIPD, an autosomal recessive disorder characterized by progressive cardiomyopathy, muscle weakness, respiratory insufficiency, and death usually by one year. **Methods:** This is a Phase 2 open-label, multinational, multicenter study of 8 patients with CIPD. Inclusion criteria are cardiomegaly by age 6 months, cardiomyopathy, CRIM(+) status and <1% GAA in fibroblasts. Chinese hamster ovary cell derived rhGAA is administered at 10 mg/kg IV weekly. Clinical efficacy assessments include ventilator-free survival, reduction in left ventricular mass (LVM), and motor development. **Results:** All patients have been enrolled (median age 4.7 months, range 2.7-14.6). rhGAA is generally well-tolerated. There have been 2 deaths, neither related to rhGAA. In 5/5 patients with 6 months of treatment data, there is reduction of LVM (mean LVM index difference from baseline to 6 months is $-157 \pm 66 \text{ gm/m}^2$, $p=0.006$; median LVM z-scores decreased from +7.4 to +2.9). After 3 months of treatment, histomorphometry of muscle glycogen content showed significant reduction in 2/7 patients evaluated so far. However, after 6 months of rhGAA therapy 6/6 surviving patients are ventilator-free and 4/6 patients have an Alberta Infant Motor score ³ 3rd percentile for age. All patients enrolled developed antibodies to rhGAA after 2 months of therapy. **Conclusions:** Preliminary data suggests that rhGAA is well tolerated and capable of improving cardiac status and skeletal muscle function in patients with CIPD. Additional data are needed to assess the long-term safety and efficacy of rhGAA, as well as the role of anti-rhGAA antibodies in clinical outcome.

A Phase I/II Study of Enzyme Replacement Therapy (ERT) for Mucopolysaccharidosis VI (MPS VI; Maroteaux-Lamy syndrome): 48 Week Progress Report. *P. Harmatz¹, C.B. Whitley², L. Waber³, R. Pais⁴, R. Steiner⁵, B. Plecko⁶, P. Kaplan⁷, J. Simon¹, J. Waterson¹, J. Hopwood⁸.* 1) Children's Hosp Oakland; 2) U Minn Med S; 3) U Tex SW Med; 4) E Tenn Children's Hosp; 5) Oregon H & S; 6) U Kinderklinik, Graz; 7) Children's Hosp Philadelphia; 8) Woman's & Children's H, Adelaide.

MPS VI is an LSD caused by a deficiency of N-acetylgalactosamine-4-sulfatase (ASB), leading to a progressive disorder with multiple organ and tissue involvement. Death usually occurs in the second decade. Currently there is no specific treatment other than symptomatic care and bone marrow transplant. A randomized, two-dose, double-blind study for 24 weeks to evaluate safety and efficacy of weekly treatment with human recombinant (rh) ASB in humans with MPS VI was recently completed. Patients were randomized to weekly infusions of either high (1.0 mg/kg) or low (0.2 mg/kg) doses of rhASB. Six patients completed at least 24 weeks of treatment (3M, 3F; age: 7-16 years); 5 patients have completed at least 48 weeks. The study blind was removed after the 24 weeks. One patient withdrew from the study for reasons unrelated to any adverse event. No study drug-related serious adverse events, significant laboratory abnormalities or allergic reactions have been observed. Complement studies were unremarkable. Patients on low or high dose rhASB had reductions in total urinary GAG excretion of 51 and 63%, respectively, at 48 weeks. Four of 5 patients had improvement in the 6 minute walk test, 3 were substantial. One of 3 patients without tracheostomy had improved forced vital capacity and two of these patients had a 4- to 7-fold decrease in Apnea-Hypopnea Index. Shoulder ROM improved in all 5 patients completing 48 week evaluations and joint pain improved in patients with significant pain. Patients on low dose enzyme were advanced to the 1 mg/kg dose after the 48 week evaluations. In conclusion, treatment was well-tolerated, urinary GAG decreased in a dose-related pattern, and clinical responses were present in all patients, but greatest in patients with advanced disease receiving high dose ERT. (Sponsored by BioMarin Pharmaceutical Inc., Novato, CA).

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Expression and Secretion of Alpha-galactosidase A by Primary Murine Myoblasts. *J. Wang, J.A Barranger.*
Human Genetics, University of Pittsburgh, Pittsburgh, PA.

Fabry disease is an X-linked inherited metabolic disorder due to a deficiency of lysosomal alpha-galactosidase A (AGA). The success of enzyme replacement for the lysosomal storage disorders, and recently Fabry disease, has stimulated consideration of alternative methods for the delivery of enzyme. One approach is a system that could supply AGA into the circulation continuously. We have previously reported that human genes coding lysosomal enzymes can efficiently transduce murine myoblasts. These cells express and secrete enzyme into the blood from which it is taken up and localized in the lysosome. We now demonstrate that primary murine myoblasts from a Fabry mouse can be transduced at high efficiency by MFG-AGA, where over 90% of MFG-AGA transduced Fabry mouse cells expressed AGA. The transduced primary murine myoblasts had intracellular enzyme activities of over 500 U/mg, while non-transduced controls had 0 activity. Furthermore, transduced primary myoblasts secrete AGA extracellularly for at least 21 weeks. Most importantly, secreted AGA was taken up by Fabry mouse cells. These data suggest that transduced primary myoblasts may be useful in supplying AGA as an alternative approach in the treatment of Fabry disease.

Enzyme replacement therapy improves sweat function and renal natural history in Fabry disease. *R. Schiffmann*¹, *K. Chen*¹, *S. Gupta*¹, *Y. Sharabi*², *R.O. Brady*¹. 1) Developmental and Metabolic Neurology Branch/NINDS/NIH, Bethesda, MD; 2) Clinical Neurocardiology Section/NINDS/NIH, Bethesda, MD.

Fabry disease is an X-linked disorder caused by a deficiency of lysosomal α -galactosidase A with accumulation of the glycosphingolipid globotriaosylceramide. Clinical manifestations include hypohydrosis with poor heat tolerance and progressive deterioration of renal function. We previously demonstrated the therapeutic safety and efficacy of enzyme replacement therapy (ERT) with agalsidase alfa for Fabry disease in a 6-month placebo-controlled trial (Schiffmann, et al, 2001). We subsequently evaluated the short, medium and long-range clinical efficacy of ERT. Patients (n=25, 19-52 yrs) received infusions of agalsidase alfa (Replagal, Transkaryotic Therapies, Inc., Cambridge, MA) at 0.2/kg/mg every two weeks for up to 3.5 years. Outcome measures were sweat function using acetylcholine iontophoresis, over 0.781 cm² of skin, for quantitative sudomotor axon reflex (QSART), renal function, and comparison with renal function data in the NIH natural history cohort (n=105, age range 11-60). When studied at the 2.5/3 year time point, pre-ERT sweat excretion in Fabry patients was 0.24 ± 0.33 mL vs. 1.05 ± 0.81 in concurrent controls (n=38, p<0.0001). Sweat function improved 24-72 hr post-enzyme infusion (0.57 ± 0.71 mL, p=0.04) and normalized in 4 anhydrotic patients. Renal function declined over 6 months in patients who initially received placebo consistent with the natural history of disease. Glomerular filtration rate significantly improved in these patients with ERT (p=0.025), and remained stable in the patient group as a whole for up to 2.5 years. No patient who has received ERT progressed to end-stage renal disease (ESRD). Time-to-event (ESRD) in treated patients was significantly better than in the NIH untreated cohort (p=0.02, log rank test). Over the last 2 years of the study, all patients have received agalsidase alfa infusions at home. Home therapy has been very well-tolerated. We conclude that ERT in Fabry disease is safe, corrects sweat malfunction, and likely beneficially alters the natural history of Fabry disease. QSART may be useful to further monitor ERT therapy.

Adenovirus-mediated transfer of huntingtin in neuronal and glial cells. *J. Shin*¹, *Z. Yu*², *S. Li*², *X. Li*². 1) Graduate Program in Genetics and Molecular Biology; 2) Dept Human Gen, Whitehead Bldg, Emory Univ, Atlanta, GA.

Despite its widespread expression, mutant huntingtin causes selective neurodegeneration in Huntington disease, an inherited disorder that results from polyglutamine expansion in the N-terminal region of huntingtin. N-terminal huntingtin containing expanded polyglutamine also forms aggregates in neurons and kills neurons in vitro. By examining HD transgenic mice expressing N-terminal mutant huntingtin, we also observed huntingtin aggregates in glial cells. Since glial cells support the survival of neuronal cells, it would be interesting to study how mutant huntingtin affects the function of glial cells and then neuronal viability. An efficient expression of mutant huntingtin in neuronal and glial cells will allow for better examining the neuropathological role of mutant huntingtin. As an initial step, we constructed adenovirus-mediated vectors that express the first 218 amino acid plus an additional 23 or 120 glutamine repeat. The expressed huntingtin is fused to the C-terminus of green fluorescence protein (GFP) to allow the visualization of transfected proteins in living cells. Western blots have confirmed that these transfected proteins are correctly expressed in HEK293 cells. Immunostaining also showed that mutant huntingtin forms aggregates or inclusions in transfected cells. We are currently preparing virus for infection of GFP-huntingtin into glial and neuronal cells and for examination of the effect of glial cells containing mutant huntingtin on the survival of neurons.

Fetal gene therapy via the cerebrospinal fluid using an HSV-1 amplicon cosmid based vector. *W. Patino¹, X.O. Breakefield², J.W. Larsen¹, A. Kwok³, C. Andry³, R.V. Lebo^{1,4}.* 1) Dept OB/GYN, George Washington Univ, Washington, DC; 2) Dept Neurology, Harvard Medical School, Charlestown, MA; 3) Dept Pathology, Boston Univ, MA; 4) Dept Pathology, Akron Childrens Hospital, Akron, OH.

Advantages of prenatal gene therapy include introducing genes to an immunologically privileged host with minimal rejection of non-self antigens, introducing normal gene function prior to progressive degeneration, and delivering genes to nerves prior to blood-brain barrier development. Advances in molecular biology and surgery make prenatal gene therapy more realistic. Proof of principle experiments tested reporter gene delivery into small laboratory animals with multiple fetuses and short gestation periods. Day 15.5 fetal mice were injected with a viral HSV-1 amplicon cosmid based vector carrying the bacterial LacZ reporter gene. During surgery, 1 microliter of packaged viral vectors carrying $>10^8$ reporter genes were delivered directly through the placenta to one of several locations including through a visible fetal fontanelle via a 50 micron I.D. glass tipped microinjection needle. The 14 dams whose injected fetuses survived for three days after surgery carried 142 fetuses for a total survival rate of 85%. Among these 142 fetuses, 81% of 47 injected fetuses survived while 86% of the uninjected littermates survived. This includes survival of 86% of fetuses injected with 1 microliter in the cerebrospinal fluid (CSF). All 18.5 day old fetuses that survived HSV-1ac injection had similar weights (1.41 0.19 grams, $P=0.13$) and crown-to-rump lengths (19.2 1.84mm, $P=0.96$) when compared to age-matched uninjected controls (1.47 0.24 grams, $P=0.16$; 19 2.79 mm, $P=0.15$) reflecting no significant adverse effect of the HSV-1ac injection in the fetal growth and development. Of the 30 fetuses screened for LacZ activity, 24 (80%) exhibited reporter gene activity in more than 3 consecutive sections. Genes introduced into the cerebrospinal fluid were expressed in multiple brain locations while fetuses injected in amniotic fluid typically expressed genes in the lung. These results suggest that gene injection into fetal cerebral spinal fluid is one approach to treat genetic neuropathies.

Metabolically biotinylated gene therapy vectors. *M.A. Barry, M.B. Parrott.* Molecular & Human Genetics, Immunology, Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX.

Viral gene therapy vectors are often handicapped by their inability to transduce therapeutically-relevant cells while avoiding transduction of non-target cells. One method of altering adenoviral specificity is to genetically introduce cell-targeting ligands directly into the capsid proteins of the vector. While this approach can work, in many cases inserted ligands fail to function or destroy the function of the virus making targeting impossible. To avoid this problem, we have developed metabolically biotinylated vectors. In these vectors, the viruses are genetically engineered to display a biotin acceptor peptide (BAP) that is enzymatically biotinylated by the endogenous biotin ligase, holocarboxylase synthetase, of the mammalian cell, or that can be biotinylated by co-expression of the bacterial ligase BirA. BAP-modification of the adenovirus fiber protein generated biotinylated virus particles that could specifically target K562 cells using biotinylated antibodies against CD59 and CD71. Flow cytometry revealed that the biotinylated vector mediated up to 95% transduction of target cells versus 5% transduction by the wild type vector. When quantitated by luciferase gene delivery, the biotinylated virus showed a greater than 200-fold increase in transduction relative to wild type Ad5. This work demonstrates the ability to produce metabolically biotinylated adenoviral vectors for vector targeting. Similar approaches are being applied for retroviral envelopes and for AAV capsids to produce a repertoire of metabolically biotinylated viruses. Optimized biotinylated vectors will take advantage of the unique dual affinity aspects of avidin where vectors can be affinity purified on monomeric avidin ($K_d = 10^{-7}$) using only biotin for release, followed by re-targeting using high affinity tetrameric avidin ($K_d = 10^{-15}$) to bridge the virions to any biotinylated targeting ligand. This approach also avoids the necessity to genetically engineer a new viral vector for every new targeting ligand, since any biotinylated ligand can be combined with a single biotinylated vector to target multiple cell types.

Suppression of mRNA Nonsense Mediated Decay in Smith-Lemli-Opitz Syndrome. *L.S. Correa-Cerro, P.A. Krakoviak, C.A. Wassif, F.D. Porter.* Heritable Disorders Branch, NICHD/NIH, Bethesda, MD.

Nonsense-mediated mRNA decay (NMD) permits cells to recognize and degrade mRNA transcripts with nonsense mutations to prevent possible toxic effects of truncated peptides. In mammalian cells, there is no consensus whether NMD takes place in the nucleus or in the cytoplasm. It has been proposed that exon-exon junctions are tagged upon completion of splicing, producing an mRNA which undergoes NMD when a premature stop codon is present at a given distance from the tagged junction. Aminoglycoside suppression of NMD has been studied in several autosomal recessive diseases such as cystic fibrosis, Tay-Sachs, and Hurler syndrome. Smith Lemli-Opitz syndrome (SLOS) is an autosomal recessive, multiple malformation syndrome due to an inborn error of cholesterol biosynthesis. We have studied four fibroblast cell lines from patients with SLOS, in which one allele has a premature stop codon: T93M/W151X (2 patients), R450L/Q98X, and IVS8-1G>C/W151X. W151X occurs in about 8% of patients, while Q98X is a novel mutation in DHCR7. To test for aminoglycoside suppression of NMD in SLOS, we treated one control and four patient fibroblast cell lines with G418 and gentamicin. After 48 hrs of treatment we isolated total RNA, performed RT-PCR and sequenced these products directly. We observed two alleles in cell lines treated with antibiotics but only one in the untreated cells. We have also observed an increase in DHCR7 expression levels by Northern analysis in the cells that underwent treatment. We are now investigating the biochemical consequences of NMD-suppression in SLOS fibroblasts. If suppression of NMD can alter the sterol profile in fibroblasts with nonsense mutations, then it may represent a therapeutic approach in this subgroup of SLOS patients.

Adenoviral and AAV vectors for an efficient intracerebral gene transfer in the Sandhoff mouse model. C.

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Sandhoff disease is an autosomal recessive neurodegenerative disease characterized by the intralysosomal accumulation of GM2 ganglioside. It is due to mutations in the HEXB gene encoding the hexosaminidases b-chain and results in a hexosaminidases A (ab) and B (bb) deficiency. In order to test the feasibility of gene transfer methods in this model, a recombinant adenoviral vector encoding the b subunit was injected intracerebrally into the murine model of Sandhoff disease. It led to a normal hexosaminidases total activity in the entire brain and a partial restoration of the Hex A specific activity. The injection of hyperosmotic concentrations of mannitol in combination with the adenoviral vector resulted in a drastic enhancement of the vector diffusion in the injected hemisphere. Although mannitol extended the inflammatory effects of the vector, the use of such a chemical agent significantly improved transduction efficiency into the CNS. AAV vectors containing the human HEXA and HEXB cDNAs under the control of the CAG promoter were also constructed. AAV-HEXB was first injected alone into the brain of hexb ^{-/-} neonates. Histological staining showed a high enzymatic activity in widely diffuse areas. Hexosaminidases activity was restored to the normal level in the whole brain, but Hex A only reached 20% of normal. The coadministration of both HEXA and HEXB vectors in the CNS permitted to reach therapeutic levels of hexosaminidases A and B, as demonstrated by the GM2 ganglioside degradation, confirming the necessity of supplying both subunits in the case of heterodimeric proteins, such as Hex A.

Towards successful gene therapy for OTC-deficiency: Evaluating *in vivo* efficacy of the Helper-Dependent

Adenoviral Vector in the OTC^{spf-ash} mouse. A. Mian¹, V. Mane¹, K. Oka², W. O'Brien¹, A. Beaudet¹, P. Ng¹, B. Lee¹.

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Ornithine transcarbamylase (OTC) deficiency, an inborn error of hepatocyte metabolism, requires a high level of hepatocyte transduction and transgene expression for therapeutic correction. We hypothesized that the helper-dependent adenoviral vector (HDV) with a tissue-restricted promoter would be able to express OTC in the hepatocyte for a prolonged period with minimal chronic toxicity. We, therefore, generated a HDV construct containing human OTC cDNA driven by the tissue-restricted phosphoenolpyruvate carboxykinase (PEPCK) promoter. To test the therapeutic potential of the HDV *in vivo*, we utilized the OTC^{spf-ash} mouse, a hypomorphic mouse model for OTC-deficiency.

Affected mice treated with 1.5×10^{11} particles of HDV:PEPCK-hOTC showed no improvement in orotic aciduria, likely because of mitochondrial leader peptide sequence differences between the human and mouse OTC proteins (Ye *et al.* Hum. Gene Ther. 2001). However, no hepatotoxicity was associated with the HDV treatment, therefore warranting further studies. To determine whether higher hOTC expression might overcome leader peptide differences in the mouse model and at the same time increase the therapeutic index in human applications, we incorporated the woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) into the adenoviral hOTC construct. Interestingly, 3×10^{11} viral particles of the HDV:PEPCK-hOTC-WPRE vector in the OTC^{spf-ash} mouse were sufficient to significantly reduce orotic aciduria for at least 6 weeks with ongoing measurements. At the same time, we generated HDV using the mouse OTC cDNA. Treatment of OTC^{spf-ash} mice with 3×10^{11} viral particles of HDV:PEPCK-mOTC corrected orotic aciduria comparable to a first generation adenoviral vector expressing the same mouse transgene. Further development of HDVs using the WPRE for *in vivo* gene replacement for diseases such as urea cycle disorders is warranted due to decreased long-term toxicity and prolonged transgene expression associated with these vectors.

TNF α signaling and its modulation in thrombocytopenia associated with adenovirus gene therapy. *V.P. Mane, G. Toietta, R. Cela, L. Pastore, A. Beaudet, B. Lee.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Intravenous administration of high doses of adenoviral vectors has been shown to be associated with thrombocytopenia leading to severe risk of disseminated intravascular coagulopathy and systemic inflammatory response. The molecular mechanism involved in the acute response leading to thrombocytopenia is not known. This early response is independent of viral gene expression, since helper-dependent Ad vectors devoid of all viral coding sequences cause a similar dose-dependent thrombocytopenia, albeit of lesser severity. To investigate a possible early response to adenoviral exposure mediated by tumor necrosis factor α (TNF α), we evaluated platelet levels following intra-tail vein administration of a first generation adenoviral vector in wild type and TNF α deficient mice on the same genetic background. The dose-dependent acute thrombocytopenia in wild type mice was more severe compared to that observed in TNF α deficient animals, indicating that TNF α plays a role in the acute toxicity associated with adenovirus. Moreover, wild type mice treated before Ad-vector injection with specific anti TNF α antibodies were partially protected from severe thrombocytopenia. Our data suggest the involvement of a host TNF α mediated response as one contributing factor leading to the dose-dependent thrombocytopenia associated with adenoviral vector administration. Moreover, a pretreatment with anti TNF α antibody may successfully decrease the acute toxicity without affecting the efficiency of transduction. The use of helper-dependent vectors in conjunction with pharmacologic modulation of TNF α signaling will increase the therapeutic index of adenovirus mediated gene therapy of genetic disease.

Gene Transfer by Receptor-Associated Protein/Polylysine Conjugates into Human Hepatoma Cells. *T. Kim¹, S. Kang¹, W. Yoo¹, S. Nam¹, J. Kang¹, M. Cho¹, H. Na¹, J. Kim¹, S. Jung², S. Kim¹.* 1) Department of Pharmacology, Korea FDA, Seoul, Seoul, Republic of Korea; 2) Korea National Institute of Health, Seoul, Korea.

The receptor-associated protein (RAP) is a ligand for all members of low density lipoprotein (LDL) receptor families and is also able to be internalized into cell via receptor-mediated endocytic trafficking, which is an attractive mechanism for efficient gene delivery. In this study, a gene delivery system using the RAP as a targeting ligand have been developed. The cDNA of RAP except C-terminal heparin binding domain was amplified by polymerase chain reaction (PCR) from human liver cDNA library and was re-amplified by primer that was added to a cysteine residue at the carboxyl end to facilitate its conjugation to polylysine (polyK), the DNA condensing non-viral gene carrier. The RAP was purified using a bacterial expression system and coupled to two kinds of polyK, poly-D-lysine (PDL) or poly-L-lysine (PLL) of average MW 50 kDa via the heterobifunctional cross-linker SPDP. Using fluorescence-labeled RAP ligand, cellular uptake of the transfection complexes into HepG2 cells was highly efficient and specific to RAP ligand conjugated with PDL. Plasmid DNA containing the luciferase reporter gene was condensed with either RAP-PDL or RAP-PLL. In vitro transfection into HepG2 cells with RAP-PDL conjugate resulted in significantly higher expression levels in comparison to non-conjugated PDL, RAP-PLL and LipofecAMINE/ DNA complexes in the presence of 10% fetal bovine serum. The maximum level of luciferase activity was detected 2 days after transfection, at which time the transfected cells were almost confluent. The degree of luciferase expression steeply declined after 3 days and persisted for at least 5 days. The transgene expression was inhibited by addition of excess RAP in the transfection medium, but augmented by treatment with Lovastatin, which inhibits cholesterol biosynthesis and increases expression of LDL receptor. These data indicates that the gene delivery is specifically mediated by LDL-receptor. Thus, this RAP-PDL conjugate could be useful as a non-viral gene delivery vector.

Phage fC31 Integrase Mediated Gene Therapy in a Mouse Model of Hereditary Tyrosinemia Type I. *P. Held¹, E. Olivares², E. Montini¹, M. Finegold³, M. Calos², M. Grompe¹*. 1) Dept Molec & Medical Gen, L103, Oregon Health & Sci Univ, Portland, OR; 2) Dept Genetics, Stanford University School of Medicine, Stanford, CA; 3) Dept Pathology, Baylor College of Medicine, Houston, TX.

Phage fC31 integrase is a site-specific integrase that mediates efficient integration of extrachromosomal DNA into the genome of the host cell using attB and attP phage attachment sites. It has been shown that phage attP sites inserted into human and mouse chromosomes at various locations can be targeted for site-specific integration by this system. In addition, "pseudo" attP sites found in human and mouse genomes can also mediate integration. These "pseudo" sites are similar, but not identical, in sequence to the native attP sites found in the bacterial genome. It has been proposed that phage integrase will be an efficient tool for non-viral gene therapy. In this study, we tested the ability of this system in the mouse model of Hereditary Tyrosinemia Type I (HTI), deficient in fumarylacetoacetate hydrolase (FAH). The full length cDNA human FAH sequence was cloned into two integrase vectors, one with an attB site (pBC-B FAH1) and one without an attB site (pBC-FAH1). Either construct was injected into FAH^{-/-} mice in a 1:1 ratio with the integrase (pCSI) or an empty vector control (pCS). The mice were removed from the protective drug, harvested, and histologically evaluated for FAH⁺ cells. Surprisingly, most FAH⁺ nodules of integrase treated liver consisted of abnormal, dysplastic cells (bizarre nuclei, enlarged cells). This phenomenon was not observed in controls (FAH plasmid without integrase) and therefore may be related to integrase overexpression. The nature of this early integrase toxicity is under investigation, but the cellular morphology suggests that integrase may be causing genomic instability. However, mice harvested after longer selection (90 days) had >75% repopulation with FAH⁺ hepatocytes that appeared healthy and of normal size. Therefore, long-term, stable correction of the FAH deficiency was possible with the integrase system despite the toxicity observed at early time-points. The copy number and structure of integrase mediated insertion events are currently under investigation.

Benefit of Cardiotrophin-1 adenoviral gene transfer in a mouse model of spinal muscular atrophy. J.C.

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Mutations of the survival of motor neuron gene (SMN) are responsible for spinal muscular atrophy (SMA), a common genetic cause of death in childhood. Although its product, the SMN protein, is ubiquitously expressed, the pathological hallmark of SMA is degeneration and loss of spinal motor neurons resulting in paralysis and muscular atrophy. We have previously generated mice carrying homozygous deletion of *Smn* exon 7 directed to neurons. Mutant mice display a dramatic and progressive loss of motor axons resulting in skeletal muscle denervation process, a constant feature found in SMA. A symptomatic therapy approach using neurotrophic factors to prevent or reduce motor neuron loss was previously proved to be efficient in other mouse models of motor neuron diseases such as the progressive motor neuronopathy (pmn) and the Super Oxide Dismutase (SOD1G93A) mice. We therefore wondered if a cardiotrophin-1 adenoviral administration (AdCT-1) might represent a therapeutic strategy in SMA. We demonstrate here that neonate intramuscular injection of AdCT-1 delays the onset of the disease, prolongs mean life span and enhances motor performances of mutant mice: the AdCT-1 treated animals show better scores on functional test like Rotarod than untreated mutants. We show that denervation process assessed by an electromyographical recording on the couple sciatic nerve gastrocnemius is less pronounced after the AdCT-1 therapy. In addition, an increased number of myelinated axons of phrenic nerve was observed in treated mutant mice indicating a protection effect of CT-1 from proximal axonal degeneration. Finally, we show that cardiotrophin-1 greatly preserves neuro-muscular junctions from abnormal accumulation of neurofilament in terminal axons, a severe pathological feature occurring in this mutant. In conclusion, we demonstrate in this work that systemic delivery of neurotrophic factor has beneficial effects in the most relevant animal model of SMA, opening promising therapeutic perspectives in SMA patients.

The Coxsackie-Adenovirus Receptor (CAR) is Highly Expressed in Metastatic Prostate Carcinoma: Potential Impact for Adenoviral Gene Therapy. *K.A. Rauen^{1,5}, R. Rodriguez², A.M. De Marzo³, P. Carroll^{4,5}, V. Weinberg⁵, F. McCormick^{5,6}.* 1) Department of Pediatrics, University of California, San Francisco, San Francisco, CA; 2) Brady Urological Institute, Johns Hopkins Hospital, Baltimore, MD; 3) Johns Hopkins Oncology Center, Department of Surgical Pathology, Johns Hopkins Hospital, Baltimore, MD; 4) Department of Urology, University of California, San Francisco, CA; 5) Comprehensive Cancer Center, University of California, San Francisco, CA; 6) Department of Microbiology and Immunology, University of California, San Francisco, CA.

Adenovirus-based gene therapy may provide an alternative mode of treatment for prostate cancer, especially for late stage and androgen independent disease for which there is currently no effective treatment. Efficient adenovirus infection of target cells depends upon the presence of the coxsackie-adenovirus cell surface receptor, CAR, which is the primary receptor for Group C adenoviruses and is important for the attachment of adenovirus to the cell membrane. To evaluate the potential efficacy of adenoviral therapy for prostate cancer, we evaluated CAR expression in normal prostate tissue, prostate carcinoma of increasing Gleason grades and metastatic disease in paraffin-embedded, archival tissues using a polyclonal antibody raised against human CAR. Immunohistochemical analysis of benign prostate epithelia demonstrated intense luminal and lateral cell membrane staining. There was a statistically significant difference in CAR membrane expression with respect to Gleason score. Metastatic prostate specimens demonstrated strong membrane staining for CAR. Adenovirus therapy may, therefore, provide an alternate modality in the treatment of prostate cancer and may be especially efficacious in the treatment of metastatic disease.

Targeted exon skipping as a promising therapeutic tool for Duchenne muscular dystrophy. *J.C.T. Van Deutekom¹, A. Aartsma-Rus¹, M. Bremmer-Bout¹, J.A.M. Janson¹, W.E. Kaman¹, E.J De Meijer¹, F. Baas², J.T. Den Dunnen¹, G.J.B. Van Ommen¹.* 1) Dept Human Genetics, Leiden Univ Medical Ctr, Leiden, Netherlands; 2) Dept Neurology, Academic Medical Ctr, Amsterdam, Netherlands.

Duchenne muscular dystrophy (DMD) is a lethal muscle disease typically caused by frame-shifting mutations in the DMD gene that abort the synthesis of the dystrophin protein. In contrast to the gene therapy studies that are based on gene replacement, we are focusing on gene correction through the targeted modulation of the splicing of the patient-specific gene. Using antisense oligoribonucleotides (AONs) directed to exon-internal sequences, we aim to induce the specific skipping of a target exon in order to generate a shorter, but in-frame transcript similar to those found in the corresponding Becker muscular dystrophy (BMD) patients, having milder phenotypes and longer life expectancies. We have recently demonstrated the feasibility of AON-induced skipping of 15 different exons within the deletion hot spot regions. In this study we show the broad therapeutic applicability of skipping these exons in cultured muscle cells from DMD-patients affected by different mutations. In fact, through the AON-mediated production of novel, in-frame transcripts, we detected dystrophin synthesis in at least 70% of treated muscle cells. We here also show the in vivo feasibility of targeted exon skipping in mouse muscle tissue. Following intramuscular injections of mouse-specific exon 46 AONs, we were able to specifically induce exon 46 skipping in a dose-dependent manner. In a subsequent time-series experiment, the skipping effect was highest at 12 days post-injection with estimated efficiencies of 20% of total RT-PCR products, and, although gradually diminishing, continued for at least 29 days. Moreover, using DMD humanized transgenic mice carrying an integrated 2.7 Mb YAC-derived copy of the human DMD gene, we were able to test human sequence-specific AONs, and induce the skipping of human DMD exons, in an experimental mouse background. Our results confirm the therapeutic potential of AON-induced exon skipping, and contribute to its further development for DMD gene therapy.

Targeted transgene expression to the airway epithelia by helper-dependent adenovirus mediated gene transfer.

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In this study we have evaluated the possibility of targeting transgene expression to the airway epithelium using helper-dependent adenoviral (HD-Ad) mediated gene transfer. We have taken advantage of an expression cassette developed for targeting transgene expression in airway epithelia and submucosal glands and used it to express transgenes in HD-Ad vectors. This expression cassette contains epithelium-specific control elements from the human cytokeratin 18 gene (K18). In order to evaluate the transduction efficiency and the level of transgene expression of HD-Ad vectors, we focused our attention on two reporter genes: one encoding a nuclear localized β -galactosidase and the other encoding the human α -fetoprotein (hAFP), a marker with potential to be used in human clinical trials due to its lack of immunogenicity. Therefore two HD-Ad vectors, one expressing NLS-LacZ and one expressing hAFP under the K18 regulatory elements were constructed (HD-Ad K18-NLSLacZ and HD-Ad K18-hAFP). Specific X-gal staining indicating expression in bronchiolar epithelium was observed three days after intranasal administration of HD-Ad K18-NLSLacZ at a dose of 5×10^{12} viral particles/kg in C56BL/6J mice. Specific epithelial expression was further confirmed by hAFP lung immunohistochemistry in mice treated with an equal dose of HD-Ad K18-hAFP. Moreover, strong hAFP expression was observed in both bronchioalveolar lavage (BAL) fluid and sera of treated mice for up to 15 weeks. Mice treated with both HD-Ad vectors did not show any severe airway inflammation as indicated by histological analysis of lung tissues. Moreover, the treatment was not associated with changes in liver function tests or thrombocytopenia. The encouraging results obtained with both the nuclear localized reporter and the secreted marker represent the basis for developing other vectors for expressing therapeutic transgenes.

Expressed multimeric hammerhead ribozymes self-cleave to monomers and abolish mutant collagen transcripts in an osteoblast model of osteogenesis imperfecta. *R. Wenstrup¹, J. Florer¹, G. Wu², I. Toudjarska³, Y. Smicum³, M.W. Kilpatrick³, P. Tsipouras³.* 1) Children's Hospital Research Foundation, Cincinnati OH; 2) Department of Medicine, University of Connecticut Health Sciences University, Farmington CT; 3) Department of Pediatrics, University of Connecticut Health Sciences University, Farmington CT.

Osteogenesis imperfecta (OI) is a heritable disorder of connective tissue whose cardinal manifestation is bone fragility. Severe variants of OI are due to dominant negative effects of mutations that do not exclude mutant chains from type I collagen molecules, a fact that must be taken into account in all strategies aimed at correcting the collagen defects in OI individuals. Antisense hammerhead ribozymes are small catalytic RNA molecules that can be targeted to any RNA molecule containing a putative cleavage site, and are thus applicable to the selective elimination of mutant gene products. RZ5-47 encodes a ribozyme designed to selectively target a truncated COL1A1 transcript stably expressed in MC3T3-E1 osteoblasts, a cellular model of OI. An expression gene containing a human COL1A1 promoter, RZ5-47 sequence, and a zeomycin resistance cassette was stably transfected into MC3T3-E1 cells; RZ5-47 was efficiently expressed and translocated to the cytoplasm. However, cleavage of mutant transcripts was equivocal, possibly due to relatively high rates of intracellular degradation of the ribozyme, as evidenced by ribonuclease protection assays (RPA) after incubations with and without cycloheximide. Subsequently, a multimeric ribozyme expression gene (M8RZ5-47) was constructed containing 8 tandemly arranged monomeric ribozyme coding sequences, with a 3' cis cleavage site. Stable transfection of M8RZ5-47 in MC3T3-E1 cells resulted in high titers of ribozyme. RPA using a multimeric ribozyme protection fragment showed self cleavage of octamers to derivative fragments. Transcripts from the mutant minigene, previously highly expressed, were completely abolished. These data demonstrates the intracellular capacity of hammerhead ribozymes to selectively cleave a target. It raises interesting possibilities for their use as therapeutic agents.

Targeted LacZ gene correction experiments in Chinese hamster ovary cells. *R.M.W. Hofstra¹, P.H.L. Schuilenga-Hut¹, M.F. Jonkman², J.P. de Boer¹, M. Nijenhuis², G. van der Steege², C.H.C.M. Buys¹, H. Scheffer¹.* 1) Medical Genetics, University of Groningen, Groningen, The Netherlands; 2) Dermatology, University Hospital of Groningen, Groningen, The Netherlands.

An increasing number of publications on gene correction has appeared in recent years. Most if not all describe the use of oligonucleotides. We have validated different methods by episomal correction of pCH110 LacZ 1651G>A in CHO-k1 cells. We failed to detect any repair of LacZ using RNA/DNA oligonucleotides, but could achieve episomal repair of at least one mutant pCH110 LacZ 1651G>A plasmid in 27% of the transfected CHO-k1 cells by using either a single-stranded or a double-stranded DNA fragment of 509 oligodeoxynucleotides. However, the percentage of corrected plasmids pooled from the corrected and uncorrected cells of the culture dish was only about 0.03%. Presence of a signature (a second silent mutation) in the corrected plasmids confirmed that the nucleotide correction was indeed achieved by means of the introduced 509 nucleotides DNA fragments. We could further optimise the repair efficiency using longer DNA fragments. With fragments of 2557 bp, 47% of the transfected CHO-k1 cells showed repair. With ODN, different kinds of mutations could be repaired, including substitutions, small insertions and small deletions. Making use of repair fragments containing five silent mutations, we showed that a region up to 165 bp can be involved in the repair process.

Inherited disorders in Amish & Mennonite Demes of Pennsylvania. *D.H. Morton¹, K.A. Strauss¹, E.G. Puffenberger¹, D.L. Robinson¹, R.I. Kelley^{1,2}.* 1) Clinic for Special Children, Strasburg, PA; 2) Kennedy Krieger Institute, Johns Hopkins, Baltimore, MD.

The Clinic for Special Children in Lancaster County PA is a non-profit medical clinic for Amish & Mennonite children who have genetic disorders. We see 38 Amish & 23 Mennonite disorders. Founder genes & drift: Most of the 61 disorders arise from mutations introduced by founders between 1700-1850 but present day disease expression rates vary. For 14 of 61 disorders, disease incidences are 1/400-500 births & carrier rates are 7-12%. More commonly disorders are clustered in a few families & disease incidence is lower. Diseases also have been excluded by drift e.g. cystic fibrosis is not seen in the Plain People of Lancaster Co. but is found in other demes. Because of different founders & a 400-year separation of the Amish & Mennonite churches, few disorders are common to both populations. Diagnosis: Most of these disorders are diagnosed by history, examination, & one laboratory test: By examination alone 13/61; Amino acid analysis 4; Organic acid analysis GC/MS 8; mutation analysis 18. Neonatal screens by tandem mass spectrometry are done routinely for 11/61 by Neo Gen, Pittsburgh. Genetic lesions are unknown for 18 disorders: 8 will be studied by candidate gene sequencing, 10 others will require linkage studies. Natural history, medical care & biology: Each disorder presents an array of clinical problems that influence disease severity. Diagnosis & management of such problems is medically important. 11/61 are lethal or invariably cause full disability. Identification of the underlying gene mutation has seldom provided insight into phenotypic diversity. Descriptions of mutations in branched chain keto acid dehydrogenase did not explain mental retardation, acute encephalopathy & brain edema of maple syrup disease. The biology of this disease can only be explained by the effects of abnormal leucine concentrations upon brain amino acid metabolism & cell volume regulation and by study of catabolic intoxication provoked by illnesses & fasts. Origins of phenotypic diversity will be most apparent to physicians who care for patients & are interested in pathophysiology & biology.

Generation of GFAP transgenic mice expressing lysosomal neuraminidase and protective protein/cathepsin A. G. Yogalingam, T. Walker, L. Mann, J. Raucci, G.C. Grosveld, A. d'Azzo. Dept Genetics, St Jude Children's Research Hospital, Memphis, TN.

Lysosomal N-acetyl-a-neuraminidase (NEU1) initiates the hydrolysis of oligosaccharides, gangliosides, glycolipids and glycoproteins by catalyzing the removal of their terminal sialic acid residues. NEU1 acquires its active and stable conformation in lysosomes by associating with the serine carboxypeptidase protective protein/cathepsin A (PPCA). Sialidosis is caused by mutations in the *Neu1* locus. The disease is systemic affecting both visceral organs and the nervous system. Knockout *Neu1*^{-/-} mice completely lack neuraminidase activity and manifest a phenotype similar to type II sialidosis. To design effective gene therapy strategies, particularly for the central nervous system (CNS) pathology, it is essential to identify the regions and cell types in the brain that have the greatest requirement for neuraminidase. We have generated transgenic mice that contain *NEU1* and *PPCA* gene sequences under the transcriptional control of an astrocyte-specific human glial fibrillary acidic protein (GFAP) promoter fragment (GNIP). In this study we describe one founder in which the GNIP transgene had integrated as head-to-tail concatamers in two independent sites; positions 7A3-BI and 14D2-3 respectively. The two transgenic alleles that were present in this original founder were segregated in subsequent matings and shown via Southern blot analysis to contain high and low copy numbers of the transgene (high TG and low TG, respectively). In low TG mice transgene expression was localized to the brain at normal levels. An unexpected and interesting finding in high TG mice was the high expression of NEU1 and PPCA transgene proteins in all tissues analyzed, including brain, liver, spleen, heart, kidney and lung. Enzyme activity measurements, Western blot analysis and immunohistochemical analysis of high TG and low TG mice will be presented. We are currently analyzing new GFAP transgenic lines. Appropriate transgenic lines are being crossed into the knockout *Neu1*^{-/-} background to determine the extent of correction of CNS pathology. (Supported in part by NIH grants DK-52025 and GM-60950).

Enzyme Replacement Therapy in Fabry Disease: Long Term Safety and Efficacy Update on a Phase 3 Study. W. Wilcox¹, D.P. Germain², M. Banikazemi³, P. Lee⁴ and Intl Fabry Study Group. 1) Cedars-Sinai Med Ctr; 2) Hôpital Européen Georges Pompidou; 3) Mt.Sinai Sch.of Med; 4) Natl Hosp. for Neurology/Neurosurg.

Fabry disease results from deficient activity of α -galactosidase A (α -GalA) and subsequent accumulation of neutral glycosphingolipids, predominantly globotriaosylceramide (GL-3). Based on results of a Phase 1/2 clinical trial (Eng et al. *Am.J.Hum.Genet.*, 68:711, 2001), a Phase 3 multicenter, multinational, randomized, double-blind, placebo-controlled trial was conducted with 58 classically affected patients receiving 1 mg/kg of recombinant human α -GalA (r-haGal, Fabrazyme®, Genzyme Corp.) every 2 weeks for 20 weeks. All 58 patients were subsequently treated with Fabrazyme in an ongoing open-label extension study for an additional 18 months. GFR has remained stable after 18-24 months of Fabrazyme treatment in the studies; average serum creatinine levels have remained stable and within the normal range during the same period of Fabrazyme treatment. Overall improvement in pain scores, assessed with the Short Form McGill Pain Questionnaire, was sustained throughout the period during which patients received Fabrazyme treatment. As expected, at the 18-month assessments, 52/58 patients (90%) IgG seroconverted; 44/52 (85%) seroconverted within 3 months of Fabrazyme treatment. This rate of early IgG seroconversion has not impacted efficacy: GL-3 reduction is maintained in multiple renal cell types examined by histopathology at up to 12 months of Fabrazyme treatment, plasma GL-3 clearance is sustained during the same period of time. Further, the majority of seroconverted patients demonstrated a four-fold or greater reduction in titer over the 18-month period. Importantly, the frequency of reactions during infusion has markedly decreased over time. This is in spite of progressive increases in the rate of administration. After 18 months of open-label treatment with Fabrazyme, 50/58 (86%) patients completed one or more complete infusions of Fabrazyme in \leq 2.5 hours, and 35/58 (60%) patients completed one or more complete infusions of Fabrazyme in \leq 2.0 hours. In summary, long-term therapy with Fabrazyme continues to demonstrate a favorable safety and efficacy profile.

Replagal in the home setting, Fabry Disease experience in the United Kingdom. *J. Stephenson, T. Hayward, G. Jones.* Healthcare at Home Ltd, Burton-upon-Trent, England.

Introduction Diagnosis of a lysosomal storage disorder can mean a lifetime of therapy. Due to lack of clinical specialists within this area, patients travel long distances for review and treatment. Difficulties of travel, associated time commitment, and limited hospital resources, impact on the quality of life of the patient and their family.

Aims The patient should be the primary manager of their chronic disease (Mellins et al 2000). Practitioners and patients should work as partners - developing strategies that give the patient the best chance to manage their disease, thereby reducing the physical, psychological, social and economic consequences of chronic illness (NIH Publication, 1997). By training patients to infuse at home, Healthcare at Home is working towards the restoration of an independent lifestyle, with an associated increase in quality of life for the patient and their family.

Methods Viewing the patient as an individual with specific needs and expectations, appreciating the uniqueness of personality, social background and experience of life (Charles-Edwards, 1983), the nurse devises a personalized training plan. The ultimate outcome being competency in the infusion process - confirmed by a pre-defined assessment tool, as approved by the multi-disciplinary team.

Results Of 145 patients diagnosed with a lysosomal storage disorder referred to Healthcare at Home over a four year period, 114, ie 79%, have been deemed competent in cannulation, reconstitution and infusion of medication at home.

Conclusions Home treatment is more convenient for the patient and their family, and can be integrated into normal daily routine. Self-administration of medication has been shown to increase self-esteem and independence (Edwards, 1995). Successful home infusion is only possible through teamwork. It is only by involving the whole team that we may afford the patient and their family the best possible care that we give (Fallowfield, 1995).

Rationale and Development of a Functional Disability Outcomes Measure for MPS I. *A.M. Skrinar, G.F. Cox.*
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Mucopolysaccharidosis Type I (MPS I) is an autosomal recessive disorder caused by a deficiency of the lysosomal enzyme alpha-L-iduronidase, which is required for the breakdown of glycosaminoglycans. The progressive accumulation of glycoaminoglycans causes clinical symptoms in many organ systems, including neurologic, respiratory, cardiovascular, skeletal, gastrointestinal, ocular, and auditory. The spectrum of MPS I-related symptoms leads to functional impairment in activities of daily living, particularly in mobility and self-care domains. No single comprehensive instrument exists to assess functional disability in MPS I patients. Specific instruments have been developed to assess the various individual clinical manifestations that can occur in MPS I disease, e.g. sleep apnea, respiratory disease, and mobility impairment. However, most were developed for use as discriminative instruments to distinguish between patients at one point in time rather than as evaluative instruments to measure differences within patients over time. Consequently, these instruments lack the sensitivity to detect meaningful clinical changes in outcomes resulting from intervention. Ceiling and floor effects may further limit the responsiveness of these instruments when the content lacks sufficient breadth to capture the wide range of functional disabilities observed in a disease such as MPS I. To overcome these limitations, we seek to develop a functional disability outcomes measure for MPS I. Patients and medical experts have been interviewed to identify domains of functional disability and to characterize specific functional limitations associated with MPS I. This information will be used to generate an initial item pool to be presented to additional patients to ensure the relevance and responsiveness of the items to the broader MPS I population. Item responses will be analyzed and revised as part of an iterative process to establish the reliability and validity of the instrument. A scoring system will be developed to increase interpretability. The MPS I questionnaire will ultimately be used to establish the burden of illness in a patient registry setting and to assess the effect of future treatments on functional disability in a clinical trial setting.

Correction of fatty acid oxidation in CPT2-deficient fibroblasts by fibrates. *J. Bastin¹, J.P. Bonnefont¹, L. Thuillier², V. Droin², N. Khadom¹, A. Munnich¹, F. Djouadi¹.* 1) INSERM U393, Hopital Necker, Paris, France; 2) Service de Biochimie B, Hopital Necker, Paris, France.

Carnitine Palmitoyl Transferase 2 (CPT2) deficiency is among the most common inherited diseases of mitochondrial fatty acid β -oxidation (FAO). Clinical phenotypes range from mild adult forms with myalgia and rhabdomyolysis to severe neonatal forms with liver failure and cardiomyopathy. It has been suggested that the severity of the disease is related to the residual enzyme activity and FAO capacity. For this reason, we hypothesized that fibrates, which can stimulate mitochondrial β -oxidation, could have beneficial effects on FAO in CPT2-deficient cells. Bezafibrate, fenofibrate, ciprofibrate, or gemfibrozil were added for 3 days in culture medium of mild-type CPT2-deficient fibroblasts, at concentration ranging from 50 to 800mM, and the oxidation rate of 3H-Palmitate (3H-Pal) was measured. Bezafibrate (BZ) induced a dose-dependant increase in 3H-Pal oxidation, with maximal effects at 800mM (3.3 ± 0.3 nmol 3H-Pal/h/mg prot vs 1.8 ± 0.2 in untreated cells; $p < 0.001$), leading to restore normal values. Other fibrates tested had no significant effects. Antimycin added to BZ-treated cells completely blocked 3H-Pal oxidation, demonstrating that 3H-Pal was entirely oxidized through the mitochondrial β -oxidation pathway. Kinetic studies run from 2h to 72h using 800mM BZ demonstrated significant increases in 3H-Pal oxidation rate starting from 6h, with maximal effects reached at 24h. Finally, we showed that BZ treatment (800mM) resulted in a 48% increase in residual CPT2 enzyme activity in deficient fibroblasts. Correction of 3H-Pal oxidation by BZ was confirmed in fibroblasts of three other patients diagnosed with mild CPT2 deficiency. However, BZ failed to increase 3H-Pal oxidation in fibroblasts from patients with severe CPT2 deficiency. These data demonstrate that pharmacological approaches based on stimulation of residual capacities can in some cases successfully restore mitochondrial FAO in fibroblasts of CPT2-deficient patients. BZ is a ligand of the peroxisome proliferator activated receptor (PPAR), and probably acts by stimulating cellular CPT2 gene expression.

a- and b-sarcoglycan delivery by recombinant adeno-associated virus: efficient rescue of muscle, but differential toxicity. *D. Dressman*^{1,2}, *K. Araishi*³, *M. Imamura*³, *T. Sasaoka*³, *L.A. Liu*⁴, *E. Engvall*⁴, *E.P. Hoffman*^{1,2}. 1) Center for Genetic Medicine, CNMC, Washington, DC; 2) University of Pittsburgh, Department of Molecular Genetics & Biochemistry, Pittsburgh, PA; 3) National Institute of Neuroscience, NCNP, Tokyo, Japan; 4) Burnham Institute, La Jolla, CA.

The sarcoglycanopathies are a group of four autosomal recessive muscular dystrophies (LGMD 2D, 2E, 2C, and 2F), caused by mutations of the a, b, g, or d sarcoglycan genes. The d-sarcoglycan deficient hamster has been the most utilized model for gene delivery to muscle by recombinant adeno-associated virus (AAV) vectors, however human patients with d-sarcoglycan deficiency are exceedingly rare with only two patients described in the United States. Here, we report construction and use of AAV vectors expressing either a- or b- sarcoglycan, the genes responsible for the most common forms of the human sarcoglycanopathies. Both vectors showed successful short-term genetic, biochemical and histological rescue of both a- and b-sarcoglycan deficient mouse muscle. However, comparison of persistence of expression in 51 injected mice showed substantial differences between AAV a-sarcoglycan (a-SG) and b-sarcoglycan (b-SG) vectors. AAV b-SG showed long-term expression with no decrease in expression for over 21 months after injection, while AAV a-SG showed a dramatic loss of positive fibers between 28 days and 41 days post-injection ($p=0.006$). Loss of immuno-positive myofibers was correlated with significant inflammatory cell infiltrate, primarily macrophages. To determine if the loss of a-sarcoglycan positive fibers was due to an immune response or cytotoxic effect of a-sarcoglycan over-expression, SCID mouse muscle was assayed for cytotoxicity after injection with AAV a- SG, AAV b- SG, or PBS. The results were consistent with over-expression of a-sarcoglycan causing significant cytotoxicity. The cytotoxicity of a-sarcoglycan, and not b- or d-sarcoglycan over-expression, was consistent with biochemical studies of the hierarchical order of assembly of the sarcoglycan complex. Our data suggests that even closely related proteins might require different levels of expression to avoid toxicity and achieve long-term tissue rescue.

Immunostimulatory Properties of Dystrophic Muscle Alter Persistence of Transgenes. *E.P. Hoffman*^{1,2}, *H. Gordish*³, *K. Araishi*⁴, *M. Imamura*⁴, *T. Sasaoka*⁴, *D. Dressman*^{1,2}. 1) Research Center for Genetic Medicine, Children's National Medical Center, Washington, DC; 2) University of Pittsburgh, School of Medicine, Department of Molecular Genetics & Biochemistry, Pittsburgh, PA; 3) University of Pittsburgh, Graduate School of Public Health, Department of Environmental and Occupational Health, Pittsburgh, PA; 4) National Institute of Neuroscience, NCNP, Tokyo, Japan.

Inherited biochemical deficits in patients with muscular dystrophy result in cycles of degeneration and regeneration of myofibers. Complementation of the biochemical deficiency in myofibers has been the major focus of experimental gene delivery in animal models of dystrophin and sarcoglycan deficiency. Recombinant adeno-associated virus (AAV) has been shown to deliver genes to mature normal muscle with long-term persistence, although persistence in dystrophic muscle has been more variable. We hypothesized that the degeneration/regeneration cycles and immune cell infiltrate in dystrophic muscle could be immunostimulatory, leading to increased immune response to a transgene relative to normal muscle. To test this hypothesis, we delivered both marker (b-galactosidase) and therapeutic genes (b-sarcoglycan) to dystrophic muscle and to normal muscle using identical AAV vectors. We show that dystrophic muscle does indeed elicit a stronger immune response to b-galactosidase, than normal muscle, showing both greater circulating antibodies ($p < 0.05$) and greater loss of transduced fibers ($p < 0.05$). Co-delivery of marker and therapeutic AAV attenuated the immune response ($p < 0.05$), despite the use of twice as much AAV. Our results show that pathological muscle is immunostimulatory towards transgene-delivered proteins, leading to decreased persistence after AAV delivery. However, biochemical rescue of myofibers by a therapeutic transgene can attenuate this immune response, and in most cases leads to immunotolerance and long-term survival of transduced fibers.

Valproic acid increases SMN levels in spinal muscular atrophy fibroblasts. *J.A. Markowitz¹, D.D. Coover², K. Schussler², A.H.M. Burghes^{2,3}, K.H. Fischbeck¹, J.P. Taylor¹.* 1) National Institute of Neurologic Diseases and Stroke, Neurogenetics Branch, Bethesda, MD; 2) Dept. of Molecular Genetics, College of Biological Sciences, Ohio State University, Columbus, OH; 3) Dept. of Medical Biochemistry, Dept. of Neurology, College of Medicine, Ohio State University, Columbus, OH.

Spinal muscular atrophy (SMA) is a severe autosomal recessive form of motor neuron disease caused by mutations leading to inadequate levels of survival motor neuron (SMN) protein. The SMN gene is normally duplicated on chromosome 5, with a telomeric copy (SMN1) that produces most functional SMN, and a centromeric copy (SMN2) that produces mainly mRNA lacking the penultimate exon. Most SMA patients harbor homozygous deletions in SMN1, limiting them to the small amount of full-length SMN produced by SMN2. Decreased SMN is associated with a decrease in the number of structures called gems formed by SMN in cell nuclei. Since the level of SMN protein is inversely correlated with phenotypic severity, agents that upregulate SMN2 expression might mitigate the severity of disease. We investigated the effects of histone deacetylase (HDAC) inhibitors on SMN transcription. Valproic acid, an approved anticonvulsant and mood stabilizer, has recently been identified as an HDAC inhibitor. We studied the effects of treatment with valproic acid on fibroblast lines from patients with SMA. Cells treated with 0.5 to 2 mM valproic acid showed increased SMN protein relative to untreated fibroblasts by Western blot. The same doses of valproate were associated with increased full-length SMN mRNA, ranging from 1.5 to 4 times untreated levels. Fibroblasts treated with 2 to 20 mM valproic acid for five days showed an increased number of intranuclear gems to several times the untreated gem number. We are currently exploring the effects of valproate in an SMA mouse model and planning in vivo studies of the effect of valproic acid on SMN expression in SMA patients. Since the doses we used overlap the range of clinically achievable serum levels, valproic acid may have therapeutic potential in SMA.

Upregulation of SMN2 gene expression by 4-phenylbutyrate: relevance for a therapeutic approach to spinal muscular atrophy. *F.D. Tiziano¹, C. Andreassi¹, C. Angelozzi¹, T. Vitali¹, E. De Vincenzi¹, A. Boninsegna², M. Villanova³, G. Neri¹, C. Brahe¹.* 1) Institute of Medical Genetics, Catholic University, Rome, Italy; 2) Institute of General Pathology, Catholic University, Rome, Italy; 3) Casa di Cura Nigrisoli, Bologna, Italy.

Spinal muscular atrophy (SMA) is an autosomal recessive neurodegenerative disorder caused by the absence of the survival motor neuron gene (SMN1) resulting in an insufficient level of the SMN protein. All patients retain at least one, usually more, copies of the SMN2 gene which produces only small amounts of functional SMN protein due to alternative splicing. Recently, data obtained in a SMA mouse model and our own independent preliminary studies have suggested that sodium butyrate could increase the synthesis of the SMN protein. We have performed a preclinical study on primary fibroblast cultures from SMA patients aimed at determining optimal conditions for stimulating SMN2 gene expression. We used 2mM 4-phenylbutyrate (4PBA) which, in contrast to sodium butyrate, showed no apparent toxic effect on cell cycle progression. Using real time PCR technology, we found that treatment with 4PBA resulted in an increase in full length SMN2 transcripts in all cell cultures except one. The maximum extent of increase in SMN2 expression varied among the different cultures and incubation times (4-16 hrs), ranging from 71% up to 350% (average: 180%) compared to untreated cultures. SMN protein expression, evaluated as number of SMN positive dot-like nuclear structures (gems), was also increased up to 2-3 fold by 4PBA treatment. Furthermore, we found that the increase in full length transcripts was not due to a reduction of the alternatively spliced form (lacking exon 7), indicating that 4PBA induces upregulation of the SMN2 gene(s) probably through its hyperacetylating activity. These results suggest that 4PBA, which is FDA approved, could be beneficial for SMA patients and will be useful for designing a therapeutic protocol.

Identification of hnRNP-G as a novel trans-factor that promotes inclusion of the alternatively spliced exon 7 of SMN2 as a basis for therapy in patients with spinal muscular atrophy. *B. Wirth, Y. Hofmann.* Dept Molecular Genetics, Inst Human Genetics, Bonn, Germany.

Proximal spinal muscular atrophy (SMA) is a common motorneuron disease caused by homozygous loss of the survival motor neuron gene (SMN1). A nearly identical copy of the gene, SMN2, fails to provide protection from SMA due to the disruption of an exonic splicing enhancer (ESE) by a single silent nucleotide exchange, which causes alternative splicing of SMN2 exon 7. Identification of splicing factors that stimulate exon 7 inclusion and thereby produce sufficient amounts of full-length SMN2 from the remained copy gene is of great importance for somatic gene therapy approaches. Here, we identify the protein hnRNP-G as a novel trans-acting splicing factor that directly binds to SMN RNA regardless of a particular RNA binding motif, and promotes the inclusion of SMN2 exon 7 in a dose-dependant manner. Moreover, hnRNP-G interacts in vivo with Htra2-b1, an SR-like splicing factor which we have previously identified to stimulate inclusion of exon 7 through a direct interaction with the AG-rich ESE in SMN2 exon 7 pre-mRNA. By use of deletion mutants of hnRNP-G we show that the interaction of hnRNP-G with Htra2-b1 rather than that of hnRNP-G with SMN pre-mRNA mediates the ESE-dependant inclusion of SMN2 exon 7. Through this assembly of hnRNP-G and Htra2-b1 on SMN pre-mRNA to a functional complex, the hnRNP protein stabilizes the SR-like protein and enhances/modulates SMN2 exon 7 inclusion. Thus, beside the overall involvement of hnRNP proteins during pre-mRNA processing, a specific function of hnRNP-G during pre-mRNA splicing can be described here for the first time. Additionally, we show for the first time that the influence of recombinant trans-acting splicing factors such as hnRNP-G and Htra2-b1 is also effective on endogenous SMN2 transcripts and increases the endogenous SMN protein to high levels.

Molecular mechanisms in chronic rejection of renal allografts. *J. Chen*¹, *M. Yang*¹, *B. Zerlanko*¹, *J.A. Tischfield*¹, *R.M. Jindal*², *A. Sahota*¹. 1) Genetics, Rutgers University, Piscataway, NJ; 2) Surgery, University of Glasgow, United Kingdom.

Advances in immunosuppressive therapy and surgical techniques have made kidney transplantation a routine procedure in most countries. One-year allograft survival rates range from 80% for cadaveric and up to 95% for living related donors (LRD). However, these advances have had little impact on chronic rejection (CR), which remains the major cause of graft loss. A wide variety of cytokines, chemokines, and growth factors have been implicated in CR but the cascades of events that eventually result in graft dysfunction are not fully understood. We used Affymetrix HG-U95A v.2 GeneChips to identify genes that are differentially expressed in CR. Data analysis tools (GeneSpring 4.2.1) were then used to organize the identified genes into functional clusters. In this preliminary study, we investigated gene expression changes in samples from transplanted kidneys that were removed from three patients because of CR. One was a cadaver transplant and the other two were LRD. The control samples were from unrelated donor kidneys pre-transplant (two cadaveric and two LRD). Experimental clustering demonstrated that gene expression profiles in the two pre-transplant LRD kidneys were remarkably similar, but markedly different from the two cadaveric donor kidneys. This may be due to the trauma associated with handling cadaveric organs and it may partly explain the lower long-term survival rates for these organs. The three kidneys removed because of CR were closely grouped with the cadaver kidneys. Fas was consistently expressed at high levels in CR. Fas, upon binding to Fas ligand, induces apoptosis, suggesting the involvement of the Fas/FasL pathway in CR. Another 201 genes functionally clustered with Fas with a correlation coefficient of 0.95. On the other hand, 128 genes (including PDGF and TGFb) were down regulated in CR. Further analyses of gene expression changes in renal biopsies or blood samples from transplant patients may identify subsets of genes that may serve as surrogate markers for the early diagnosis of CR.

Adenoviral Rescue of Mice Deficient for Succinate Semialdehyde Dehydrogenase (SSADH; ALDh5a1^{-/-}). *M. Gupta¹, M. Callan¹, H. Senephansiri¹, B.M. Hogema^{1,2}, C. Jakobs², M. Grompe¹, K.M. Gibson¹.* 1) Molecular and Medical Genetics, Oregon Health & Science University, Portland, OR, USA; 2) Metabolic Unit, Dept Clin Chem, VU Med Ctr, Amsterdam, Netherlands.

SSADH deficiency (OMIM 271980), presenting as gamma-hydroxybutyric (GHB) aciduria, is a rare autosomal recessive defect of GABA degradation featuring non-specific neurological manifestations including psychomotor and language delays, and epilepsy. Aldh5a1^{-/-} knockout mice manifest a similar phenotype but die in the first month of life from repetitive tonic-clonic seizures. Previous work demonstrated that mutant mice could be pharmacologically rescued with the antiepileptic vigabatrin, CGP 35348 (a GABA_B receptor antagonist), NCS-382 (a GHB receptor antagonist), and the non-protein amino acid taurine. Aldh5a1 is highly expressed in liver; thus, we hypothesized that adenovirus administration (i.p.) directed towards liver could rescue mice from premature death. Plasmid pAd:RSV (kindly provided by Dr. B. Fang) was derived from pCX1 and substituted with Aldh5a1 E1 sequences, driven by an RSV promoter with BGH poly A tail. Following co-transfection into 293 cells with adenovirus plasmid pJM17, an E1 defective recombinant Adv-pAd:RSV:Aldh5a1 was generated via homologous recombination. Aldh5a1^{-/-} mice were injected i.p. with 10⁸ pfu at day 0 or 10 post-natal. Survival to day of life 30 was scored as significant. Seven of 18 injected mice survived (39%; p<0.001). Limited analysis of mice injected at day 0 revealed similar results (1/3 survivors; 33%). Despite lowered body weight, treated mice appeared healthy. Aldh5a1 enzyme activity from liver (harvested at day of life 35-50; day 10 injected animals only) was undetectable, most likely related to temporal loss of expression. Hepatic correction of Aldh5a1 deficiency may represent a new approach for treatment of inherited SSADH deficiency, historically considered a predominantly neurometabolic disorder. Supported by NIH NS40270 and March of Dimes #1-FY00-352.